The Concurrent Anti-C1q and Anti-dsDNA but not Anti-C3b Antibodies as Potent Biomarkers for Lupus Nephritis prediction

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Abstract

Background: Lupus Nephritis (LN) in patients with Systemic Lupus Erythematosus (SLE) is one of the most serious and prevalent manifestations. The procedure of renal biopsy is harmful and accompanied by potential hazards. Therefore, introducing reliable biomarkers to predict LN is exceedingly worthwhile. In the current study, we compared the diagnostic values of circulating autoantibodies against dsDNA, C1q, C3b, SSA, SSB, and Sm alone or in combination to predict LN.

Methods: This study evaluated abovementioned autoantibodies in 40 healthy controls (HCs) and 95 SLE patients with different kidney involvements, including absent (n = 40), inactive (n = 20), and active (n= 35) LN using EIA method.

Result: The frequency and odds ratio of anti-dsDNA (71.4%, OR=4.2), anti-C1q (62.9%, OR= 5.1), and the simultaneous existence of anti-C1q and anti-dsDNA (51.4%, OR=6) antibodies were significantly higher in the active LN group compared with both inactive and absent LN groups. Moreover, the levels of anti-C1q and anti-dsDNA antibodies positively correlated with disease activity in patients with SLE. The prevalence of these autoantibodies was associated with the severity of LN biopsies.

Conclusion: These data suggest that anti-C1q and anti-dsDNA antibodies and also the simultaneous presence of them may be valuable diagnostic biomarkers for LN prediction in patients with SLE.

Highlights

The key messages (highlights) of this study including:

Limited studies have been performed to evaluate the simultaneous presence of anti-complements and other pathogen antibodies and also their association with lupus nephritis severity in patients with SLE disease with different kidney involvements. In this study, in addition to healthy control, the patients with no history of nephritis were compared with SLE patients with active and inactive nephritis. Finally, the results emphasized the importance of simultaneous screening of anti-C1q and anti-dsDNA antibodies in patients with SLE to predict nephritis.

Background

Systemic Lupus Erythematosus (SLE) is a prototype of autoimmune diseases identified by the generation of numerous autoantibodies in addition to multiple tissue and organ involvements. Lupus Nephritis (LN), occurs in more than two-thirds (> 70%) of all patients with SLE, is a severe complication and it can lead to kidney impairment in many cases who are not controlled by the treatment [1, 2]. Therefore, early diagnosis of LN or prediction of the risks to develop kidney involvement have great clinical importance in the management of inhibition therapy for SLE patients [3]. So far, more than one hundred and eighty pathogenic and non-pathogenic autoantibodies have been identified in different SLE phenotypes; this variety of the autoantibody spectrum is unique among autoimmune diseases [4]. These autoantibodies target nuclear, cytoplasmic, and cell surface antigens.

Autoantibodies binding double-stranded deoxyribonucleic acid (anti-dsDNA) are the prominent hallmark of SLE. At the moment, anti-dsDNA antibodies are one of the chief suspects that directly provoke the autoimmune pathogenesis in glomeruli [4, 5]. However, antibodies against dsDNA were detected in a large number of patients with SLE lacking kidney involvement or having inactive LN [6–8]. Additionally, other conventional parameters such as antinuclear antibodies (ANAs), anti-Sm, anti-SSA or anti-complement antibodies, urine sediments, proteinuria, and a reduction in C4 and C3 complement levels have not been proposed as highly specific biomarkers for the prediction of kidney involvement, which results in exploring other novel and reliable biomarkers [9].

The antibodies against dsDNA are considered sensitive and specific biomarkers for SLE concerning the high frequency ranging from 60% to more than 95% depending on different clinical manifestations. Identification of antibodies against dsDNA serves as a diagnostic marker to SLE because the presence of these autoantibodies is rare in other autoimmune diseases and healthy individuals (almost less than 0.5%) [6, 10, 11]. There is a bulk of evidence revealing the pathogenic role of serum with anti-dsDNA antibodies and kidney involvement. Data from these studies demonstrated the deposition of these antibodies in various kidney structures, including tubules, glomeruli, mesangium, basement membrane, subendothelial, and surrounding spaces. In this regard, data from the literature indicated that the high titer of anti-dsDNA could develop LN and positively correlated with the activity of nephritis [12, 13]. Contrary to the abovementioned findings, a few numbers of the patients with SLE and also LN, ranging from 5–30%, has no detectable circulating anti-dsDNA antibodies [6, 14].

Recently, autoantibodies against serum molecules, such as complement components, have been broadly explored. For the first time in 1971, it was reported that sera from patients with SLE involved molecules binding C1q. Thirteen years later, Uwatoko et al. recognized these molecules as IgG antibodies that targeted the collagen-like regions of C1q [15, 16]. Until now, the collagen-like regions are considered the major epitopes targeted by antibodies against C1q in the sera of SLE patients. In particular, these epitopes were isolated from the renal glomeruli of SLE patients with nephritis [17]. The actual prevalence of antibodies against C1q molecules is controversial in different kidney involvements of patients with SLE. Several studies have addressed antibodies against C1q as a potential biomarker for the prediction of kidney involvement [18–20]. However, few investigations revealed the positive serum for anti-C1q directly correlated with only SLE disease activity and it is not specific for kidney involvement (LN) [21, 22]. Common pathways of the complement system, including alternative, classical, and mannose-binding lectin (MBL) are convergent by the C3 component. Autoantibodies against C3b have been reported in sera of patients with SLE and few autoimmune diseases such as Crohn disease and C3 Glomerulopathy. There is very little research associated with the prevalence of IgG antibodies against C3b molecules in patients with SLE. Interestingly, these autoantibodies were related to active LN and could be beneficial for monitoring LN activity [23–26].

In the current study, we screened circulating anti-C1q, anti-C3b, and anti-dsDNA IgG antibodies in 95 Iranian SLE patients with different kidney involvements. Furthermore, we investigated the simultaneous presence of these antibodies with other important autoantibodies, including anti-SSA, anti-SSB, and anti-Sm IgG antibodies to identify sensitive and specific diagnostic biomarkers for LN prediction. Finally, we studied whether these IgG antibodies correlate with the development and severity of LN.

Methods

Study Population: In this study, 40 healthy controls (HCs) and 95 patients with SLE participated from September 2017 to December 2018 at the Rheumatology and Nephrology Departments of Rasoul-Akram Hospital, Tehran, Iran. All of the patients and HCs filled and signed written informed consent. The revised American College of Rheumatology (ACR) criteria and Kidney Disease: Improving Global Outcomes (KDIGO) criteria for glomerulonephritis were used to diagnose and classify patients with SLE and LN, respectively. Disease activity was determined based on the SLE Disease Activity Index (SLEDAI) criteria. The patients group were categorized into three subgroups, according to kidney involvement: absent, inactive, and active LN. Kidney involvement was defined as laboratory and clinical manifestations. The SLE patients without a history of renal involvement and normal proteinuria (less than 150 mg/day) for at least five years were determined as the absent LN group (n = 40). The SLE patients with normal urinary sediment and stable kidney function with less than 0.5 g per day proteinuria were defined as inactive LN group (n = 20). Finally, the active LN group (n = 35) was defined as biopsy-proven LN with proteinuria greater than 0.5 g per day or active urinary sediment, including white and red blood cell casts, hematuria, and leukocyturia. Biopsy proved LN condition was categorized

based on the guideline of the International Society of Nephrology/Renal Pathology Society (ISN/RPS). This study was in compliance with the principles of the Declaration of Helsinki and the Ethics Committee for the performance of Human Research of Iran University of Medical Sciences (940330 – 26574). Serum (4 mL) and urine (24 h) samples of 95 SLE patients and 40 HCs were collected. Immediately, routine laboratory tests were performed and all sera were frozen in 250 μL aliquots at -80 °C for autoantibody detection.

Measurement of laboratory parameters and autoantibodies:

We evaluated serum creatinine levels and C3 and C4 complement components using Jaffe and immunoturbidimetric automated methods according to the manufacturer's instructions, respectively (Roche Diagnostics, Cobas 6000 analyzer, Germany). Trichloroacetic acid (TCA) precipitation method applied to measure urine protein. Antibody against dsDNA was assayed using an indirect ELISA kit (EUROIMMUN, Germany). The positive results were reconfirmed by indirect fluorescent assay (IFA) technique on Crithidia luciliae substrate slides (nDNA, Generic assay, GMBH, Germany) according to the manufacturer's instruction. Anti -SSA, -SSB, and -Sm antibodies were measured by indirect ELISA (AESKU. Group, Diagnostics, Germany).

In brief, standards and diluted sera were added to the antigen pre-coated wells and incubated at 25 °C for 30'. Following three times wash, anti-human IgG conjugated with Horseradish Peroxidase (HRP) was added to each well and incubated at 25 °C for 30'. After another wash, the chromogenic substrate was added and the plates were incubated at 25 °C for 20'. Then, by adding stop solution, the reactions were stopped and the absorbance of all wells was read at 450 nm by ELISA reader (BP 800, Finland).

Antinuclear antibodies (ANAs) were measured by an indirect immunofluorescent assay (IFA) technique with Human epithelial type 2 (HEp-2) cells as the substrate (Generic assay, GMBH, Germany). In brief, 25 µL of diluted sera (1:80) were added to the HEp-2 cell-substrate slides and incubated in a humidified chamber at 25 °C for 30'. The slides were softly rinsed and three times washed with Phosphate-buffered saline (PBS) at 25 °C for 20'. The slides were stained with anti-human IgG antibody conjugated with Fluorescein isothiocyanate (FITC) at 25 °C for 30' (protected from light). After three times wash, the slides were assessed on the fluorescent microscope and ANA patterns of the positive samples were reported. Remarkably, based on the manufacturer's instruction, the cut-off point was considered the titer more than 1:80.

Detection of anti-complement antibodies: Antibodies against C1q and C3b were measured using enzyme immunoassay (EIA). In summary, 10 mg/mL of C3 and C1q complement proteins (Complement Technology, USA) in 0.1 M sodium carbonate buffer (pH 9.6) were coated separately in 96-well plates (ELISA MaxTM, BioLegend, San Diego, USA) overnight at 4 °C. The plates were washed three times with PBS-T (0.05% Tween-20 in PBS) as a wash buffer. The wells were incubated with PBS containing 3% Bovine Serum Albumin (BSA) as blocking buffer at 4 °C for 2 h. After three times washing with PBS-T, prespun sera were diluted (1:50) in PBS and incubated at 25 °C for 1 h. The plates were washed five times and anti-human IgG conjugated to HRP enzyme (Invitrogen, California, USA) was added at 25 °C for 1 h. The enzyme activity of HRP was assayed by the addition of tetramethylbenzidine (TMB) substrate solution to each well and incubated at 25 °C for 30' (protected from light). Hydrochloric acid 1 N (HCl) was added to each well to stop reactions. Instantly, the absorbance of each well was read at 450 nm versus 630 nm (as a reference filter) by microplate ELISA reader (Biohit, BP 800, and Finland). For anti-C1q antibody detection, the diluent buffer for the sera and also secondary anti-human IgG (HRP-conjugated) included 1.0 M NaCl in PBS (High Salt PBS). This diluent removes the non-specific binding of anti-C1q antibody and collagen-like regions of C1q molecules. The OD records were standardized in unit relative comparison with reference sera. The values of reference sera were defined as SLE sera with high anti-C1q and anti-C3b levels corresponding to 1000 arbitrary units (AU). Each sample was tested in duplicate within an experiment and all experiments were carried out twice and also the reference sera were applied to each experiment.

Using Receiver operator characteristic (ROC) analysis, the cut-off points were determined as Youden Index that included the highest sensitivity and specificity of AU [23, 27].

Statistical Analyses

Laboratory and clinical data were analyzed and presented by SPSS version 23 (SPSS Inc, Chicago, USA) and Prism version 6.03 (Graph Pad Software, CA, USA), respectively. Statistical assessment was performed among a single HC group and three SLE subgroups. Data were presented as frequency (%) and the mean ± SD. Quantitative variables were analyzed by T-Test (Student's T-Test), one-way ANOVA test, and bivariate Pearson's Correlation Analysis. The association and odds ratio between qualitative variables were analyzed by chi-square (or Fisher's exact) test. Moreover, ROC analysis was used to characterize the area under curve (AUC) and the best cut-off points for certain variables. In all tests, a *p*-value of < 0.05 was statistically considered to be significant.

Results

This study contained 95 patients with SLE in three subgroups based on kidney involvement. The mean ± SD age of the patients with SLE and HCs was 35.9 ± 11.65 years (range 18 to 71) and 36.2 ± 11.3 years (range 19 to 55), respectively. There were no significant differences in terms of age, gender, and also the duration of disease among the absent-LN, inactive-LN, and active-LN subgroups (age, gender, and disease duration were matched). The serum levels of C3 and C4 complements were significantly lower in the active-LN group than the absent-LN group ($p < 0.001$ and $p = 0.036$) and also HCs ($p < 0.001$). However, only C3 levels were significantly decreased in active LN compared with inactive LN ($p = 0.018$). In addition, the active LN group showed worse kidney function with higher eGFR and proteinuria than inactive-LN, absent-LN, and HCs. The ANA positive results were more frequent in absent LN (65%) than inactive (45%) and active (57%) LN groups. Furthermore, most ANA patterns in SLE patients were homogenous for active LN (65%) and speckled for absent (79%) and inactive (63%) LN groups. Demographic data, clinical history, and laboratory findings of the HCs and patients with SLE are shown in Table 1.

Table 1
Demographic, clinical, and laboratory data of the SLE patients and healthy controls

Clinical and laboratory findings	HCs (n = 40)	SLE		
		Absent LN (n = 40)	Inactive LN (n = 20)	Active LN (n = 35)
Age, y	36.2 ± 11.3	37.3 ± 12.5	37 ± 15.1	33.6 ± 7.7
Gender, Female/Male	34/6	33/7	15/5	28/7
White Blood Cell, 10^3/μL	5.7 ± 1.8	6.1 ± 2.1	5.9 ± 1.9	7.1 ± 2.9
Hemoglobin, g/dL	13.5 ± 1.4	12.7 ± 1.3	11.7 ± 2.1	10.6 ± 2.8
Mean cell volume, fL	86 ± 6.1	85.4 ± 7.4	84.4 ± 8.1	79.7 ± 20.1
Platelet, 10^3/ μL	241 ± 93.9	237 ± 88.5	230 ± 76.1	227 ± 103.4
Serum creatinine (mg/dL)	0.82 ± 0.13**	0.87 ± 0.14**	1.03 ± 0.17*	2.1 ± 2.5‡
C3 (mg/dL)	134 ± 20.6**	125.5 ± 30.1**	120.9 ± 31.1**	102.9 ± 36.3‡
C4 (mg/dL)	32.7 ± 9.8**	21.6 ± 8.3*	17.4 ± 4.1	16.5 ± 9.5‡
ESR (mm/hr)	9.1 ± 5.4**	22.8 ± 16.7**	25.8 ± 15.3*	46 ± 26.1‡
eGFR (mL/min/1.73 m2)	106.2 ± 18.9**	101.5 ± 20.1**	87.3 ± 20.4*	73.2 ± 32.1‡
Proteinuria (mg/day)	< 150	< 150	368.8 ± 130.2*	1164.6 ± 1041‡
Disease duration	-	9.8 ± 3.3	8.4 ± 5.3	8.1 ± 6.2
SLEDAI	-	6.93 ± 3.3*	8.3 ± 3.7*	19.1 ± 4.1
ANA, Positive (%)	-	26 (65)	9 (45)	20 (57)
Pattern: Speckled/Homogenous, (%)		79/21	66/34	35/65

Abbreviation: ANA: antinuclear antibody, eGFR: estimated glomerular filtration rate (was calculated by the MDRD equation), C3: complement component 3, C4: complement component 4, HCs: healthy controls, LN: lupus nephritis, SLE: systemic lupus erythematosus, SLEDAI: SLE disease activity index. *p < 0.05, **p < 0.01 compared to active LN; †p < 0.05, ‡p < 0.01 compared to HCs. Values are expressed as mean ± SD and percentage.

Screen For Autoantibodies

The frequency of IgG antibodies detected in the sera of patients with SLE was as follows: anti-dsDNA (56%), anti-SSA (45%), anti-C1q (43%), anti-C3b (34%), anti-Sm (28%), and anti-SSB (21%). Some of these autoantibodies significantly differed among the SLE subgroups. A higher prevalence of IgG antibodies against dsDNA, C1q, and C3b was observed in active and inactive LN groups than absent LN group. However, there were no significant differences across SLE subgroups in terms of the prevalence of anti-Sm, anti-SSA, and anti-SSB antibodies. The best odds ratio (OR) for the prediction of active LN were anti-dsDNA (OR = 4.2, $p = 0.003$), anti-C1q (OR = 5.1, $p < 0.001$), and concurrent anti-C1q and anti-dsDNA (OR = 6, $p = 0.001$) autoantibodies. Interestingly, concurrent anti-C1q and -dsDNA (51%), as well as anti-C3b and anti-dsDNA (34%) autoantibodies, were more frequent in active-LN than other subgroups. The distribution of the simultaneous presence of antibodies against dsDNA, C1q, and C3b was higher in active-LN patients (68.6%) than SLE

patients without kidney involvement (22.5%). The frequency, *p*-value (*p*), and OR with 95% confidence interval (CI) of detected autoantibodies are listed in Fig. 1 and Table 2.

Table 2
Prevalence of autoantibodies among SLE patients with different kidney involvement.

Autoantibody	SLE (n = 95)	Absent LN (n = 40)	Inactive LN† (n = 20)			Active LN† (n = 35)		
	+ve (%)	+ve/-ve	+ve/-ve	p	OR (95%CI)	+ve/-ve	p	OR (95%CI)
Anti-dsDNA	53 (56)	15/25	13/7	**0.044**	**3.01 (1.1–9.48)**	25/10	**0.003**	**4.2 (1.6–11)**
Anti-C1q	41 (43)	10/30	9/11	0.116	2.4 (0.79–7.6)	22/13	**0.001>**	**5.1 (1.9–13.7)**
Anti-C3b	32 (34)	9/31	7/13	0.3	1.86 (0.57-6)	16/19	**0.033**	**2.9 (1.1–7.8)**
Anti-Sm	27 (28)	9/31	5/15	0.83	1.15 (0.33-4)	13/22	0.16	2 (0.7–5.6)
Anti-SSA	43 (45)	16/24	9/11	0.71	1.23 (0.41–3.6)	18/17	0.32	1.6 (0.6–3.9)
Anti-SSB	20 (21)	10/30	4/16	0.38	0.53 (0.13–2.2)	6/29	0.41	0.6 (0.2–1.9)
Anti-dsDNA + Anti-C1q	31 (33)	6/34	7/13	0.1	3 (0.9–10.8)	18/17	**0.001**	**6 (2-17.9)**
Anti-dsDNA + Anti-C3b	20 (21)	5/35	3/17	0.99	1.2 (0.3–5.8)	12/23	**0.03**	**3.65 (1.1–11.7)**
Anti-C1q + Anti-C3b	20 (21)	6/34	4/16	0.7	1.4 (0.35–5.7)	10/25	0.25	2.3 (0.7–7.1)
Anti-dsDNA + Anti-C1q + Anti-C3b	14 (15)	4/36	2/18	0.99	1 (0.17–5.98)	8/27	0.23	2.7 (0.7–9.8)

Abbreviation: +ve/-ve, Positive/Negative. † OR was compared with absent LN group. Significant *p* values shown in bold

The ROC curve analysis revealed that only antibodies against dsDNA and C1q were considered better biomarkers of active-LN with higher AUC, sensitivity, and specificity. However, only anti-dsDNA antibodies were considered a significant biomarker for inactive LN group (Fig. 2A and Table 3). The SLEDAI scores were significantly higher for SLE patients with positive anti-dsDNA (13.5 ± 6.6 vs. 7.7 ± 5.1) and anti-C1q (14.7 ± 6.5 vs. 9.4 ± 5.9) antibodies ($p < 0.001$). Importantly, there was no difference for SLEDAI scores between positive anti-C1q and anti-dsDNA antibodies. Serum levels of C3 and C4 were significantly lower in SLE patients with positive anti-C3b and anti-dsDNA antibodies versus the negative ones (Fig. 2B, C,D and E).

Table 3
Sensitivity, specificity, AUC, and predictive value of autoantibodies in active and inactive LN groups.

Autoantibody	Inactive LN (n = 20)						Active LN (n = 35)					
	p Value	AUC (95%CI)	SEN	SPE	PPV	NPV	p Value	AUC (95%CI)	SEN	SPE	PPV	NPV
Anti-dsDNA	**0.08**	**0.64 (0.49–0.79)**	**65**	**62.5**	**46**	**77**	**0.003**	**0.7 (0.58–0.82)**	**71.4**	**62.5**	**62.5**	**71**
Anti-C1q	0.2	0.6 (0.44–0.76)	45	75	47	73	**0.005**	**0.73 (0.61–0.85)**	**62.9**	**75**	**69**	**67**
Anti-C3b	0.43	0.56 (0.4–0.72)	35	77.5	43	70	0.8	0.62 (0.49–0.74)	45.7	77.5	64	62
Anti-Sm	0.9	0.53 (0.34–0.69)	25	77.5	36	47	0.28	0.57 (0.44–0.7)	37.1	77.5	59	58.5
Anti-SSA	0.75	0.5 (0.37–0.68)	45	60	36	69	0.39	0.56 (0.43–0.69)	51.4	60	53	59
Anti-SSB	0.74	0.52 (0.38–0.67)	20	75	29	65	0.54	0.56 (0.4–0.68)	17.1	75	37.5	51

Abbreviation: AUC: area under curve, NPV: negative predictive value, PPV: positive predictive value, SEN: sensitivity, SPE: specificity. Sensitivity, specificity, PPV, and NPV were presented with percentage. Significant *p* values shown in bold.

The levels of antibodies against dsDNA positively correlated with anti-C1q antibodies ($p < 0.01$, $r = 0.3$), ESR ($p < 0.01$, $r = 0.28$), and SLEDAI score ($p = 0.02$, $r = 0.23$), but negatively correlated with serum levels of C4 ($p < 0.01$, $r = -0.46$) and C3 ($p < 0.01$, $r = -0.38$). Of note, the levels of antibodies against C1q in addition to anti-dsDNA antibodies significantly correlated with antibodies against C3b ($p < 0.01$, $r = 0.36$), SLEDAI score ($p < 0.01$, $r = 0.42$), ESR ($p < 0.01$, $r = 0.3$), and serum levels of C4 ($p < 0.01$, $r = -0.31$). Furthermore, anti-C3b antibodies showed a negative correlation with the levels of C3 ($p = 0.02$, $r = -0.21$) and C4 ($p < 0.01$, $r = -0.25$).

The most common class of LN based on ISN/RPS was class IV (45.7%), followed by class III (25.7%), class II (14.3%), class V (11.4%), and then class VI (2.9%). The frequency of anti-C1q and anti-dsDNA was superior to the other

antibodies in all classes of kidney biopsies. Particularly, these frequencies were higher (n = 12, 75%) in class IV of histological nephritis (Table 4). Importantly, the negative serum for anti-Sm and anti-SSB antibodies were protective for severe class IV of LN. More importantly, the frequency of simultaneous presence of anti-dsDNA/anti-C1q (n = 6, 17.1%), anti-dsDNA/anti-C3b (n = 5, 14.3%), anti-C1q/anti-C3b (n = 5, 14.3%), and anti-dsDNA/anti-C1q/ anti-C3b (n = 5, 14.3%) were higher in the class IV group than other groups. Data are shown in Table 4.

Table 4
Distribution of autoantibodies in different kidney biopsies based on ISN/RPS.

Autoantibody	Kidney biopsy†				
	Class II (n = 5)	Class III (n = 9)	Class IV (n = 16)	Class V (n = 4)	Class VI (n = 1)
Anti-dsDNA	4 (11.4)/1(2.9)	5(14.3)/ 4(11.4)	**12**(34.3)/**4**(11.4)*	3(8.5)/1(2.9)	1(2.9)/0(0)
Anti-C1q	3(8.5)/2(5.7)	4(11.4)/5(14.3)	**12** (34.3)/**4**(11.4)*	3(8.55)/1(2.9)	0(0)/1(2.9)
Anti-C3b	3(8.55)/2(5.7)	5(14.3)/4(11.4)	6(17.1)/10(28.6)	1(2.9)/3(8.55)	1(2.9)/0(0)
Anti-Sm	3(8.6)/2(5.7)	5(14.3)/4(11.4)	**4**(11.4)/**12**(34.3)*	0(0)/4(11.4)	1(2.9)/0(0)
Anti-SSA	3(8.55)/2(5.7)	4(11.4)/5(14.3)	10(28.6)/6(17.1)	1(2.9)/3(8.55)	0(0)/1(2.9)
Anti-SSB	**0**(0)/**5**(14.3)*	**1**(2.9)/**8**(22.8)*	**4**(11.4)/**12**(34.3)*	1(2.9)/3(8.5)	0(0)/1(2.9)

Abbreviation: +ve (%)/-ve (%): positive (%)/negative (%). †:The mixed classes were counted in the higher class, *: significant p values shown in bold ($p < 0.05$).

Discussion

We evaluated the prognostic and diagnostic value of circulating anti-dsDNA, anti-C1q, and anti-C3b antibodies alone or in combination with other autoantibodies in SLE patients with different kidney involvements. The results indicated that antibodies against dsDNA and C1q were more frequent in active and inactive LN groups. In this regard, the prevalence of them was higher in active LN patients. Moreover, disease activity (SLEDAI scores) was higher for the patients having these autoantibodies. Conversely, C3 and C4 levels were lower for patients having anti-dsDNA and anti-C3b antibodies, while no significant differences were observed in patients with positive antibodies against C1q. Importantly, the frequency of anti-dsDNA and anti-C1q was superior to the other antibodies among histological classes of kidney biopsies. Of note, more than 75% of SLE patients with severe LN (Class IV) have anti-dsDNA or anti-C1q antibody.

These data indicated that the evaluation of the simultaneous existence of antibodies against dsDNA and C1q could be useful as diagnostic and predictive biomarkers for SLE patients with kidney involvement. Several studies are consistent with our findings [5, 12, 18, 20]. Recently, it has been demonstrated that any deficiency in complement activation through classic and alternative pathways strongly correlated with the progression and severity of LN. Among these complement components, C1q and C3b are the most widely explored factors and have a crucial role in facilitating the clearance of immune complexes and cell residue [9, 28]. In this regard, the production of antibodies against C1q may result in the formation of circulating or local complexes of C1q and anti-C1q antibodies. These complexes may deposit in the kidneys and lead to tissue damage. Moreover, anti-C1q may induce a pathogenic condition via disrupting the clearance activity of apoptotic cells, triggering autoimmune inflammatory responses [17, 22].

Although a clinical significance has been observed between the existence of anti-C1q antibodies and LN, the diagnostic value and pathogenic role of antibodies to C1q in LN remains controversial [21, 29]. Anti-C1q autoantibodies may be

found in the sera of healthy individuals and patients with other autoimmune diseases such as rheumatoid vasculitis, mixed connective tissue disease (MCTD), Felty syndrome (FS), and autoimmune thyroid disease [22, 30, 31]. Particularly, these antibodies are detected at a high titer almost in all patients with hypocomplementemic urticarial vasculitis (HUV) syndrome [32, 33]. Available data suggest that anti-C1q antibodies on pathophysiological levels are essential, but not enough for beginning the immune inflammation in the renal glomeruli since some healthy persons and patients with UHV syndrome with high levels of anti-C1q antibodies do not experience kidney involvement throughout their lives [21, 30, 33].

Furthermore, Flierman et al. indicated that animal model experiments with the injection of anti-C1q antibodies or anti-glomerular basement membrane (GMB) alone did not lead to obvious kidney disease; however, the simultaneous injection of them is necessary for inducing kidney inflammation [34]. Nevertheless, several studies have shown the deposition of anti-C1q antibodies and C1q molecules along with tubular and glomerular basement membranes [35].

In the current study, we found that antibodies to dsDNA were the most prevalent autoantibody in patients with SLE. Furthermore, this frequency was higher when patients with SLE were classified based on kidney involvement with active (71.4%) and inactive (62.5%) LN. In this context, Fabrizio and colleagues categorized patients with SLE, according to the presence of anti-dsDNA antibodies, including persistent negativity (24.4%) and positivity (62.3%) for anti-dsDNA as well as patients with initial positivity and further negativity during the disease course (13.3%). In agreement with our data, they identified that kidney involvement was significantly more frequent in patients with SLE having anti-dsDNA antibodies [6]. Moreover, we showed the C4 and C3 levels were lower in patients with the positive anti-dsDNA antibody. The formation of immune complexes as a result of autoantibodies can fix complement proteins and induce systemic inflammation responses. The kidneys are one of the major sites of these inflammatory processes because deposited anti-C1q and anti-dsDNA antibodies are enriched in kidney elutes and its levels correlate with the severity of nephritis. In summary, the unique ability of these autoantibodies to induce kidney injury alone comprises the formation and deposition of circulating immune complexes in the kidney glomeruli, binding directly to kidney antigens, and induction of inflammatory cytokine afterward [5, 36, 37]. Consequently, evaluation of the simultaneous presence of antibodies against C1q and dsDNA can enhance the predictive power of detection of patients with active LN.

Recently, a few studies have revealed that anti-Sm antibodies are associated with the early poor outcome of LN [38, 39]. Accordingly, Ishizaki et al. [39] showed high levels of autoantibodies against Sm may predict silent LN without abnormal proteinuria. Notably, in our study, we did not recognize any significant association among the frequency of antibodies against SSA, Sm, and SSA with the severity of the disease (SLEDAI), serum complement levels, and kidney involvement. A possible description for this might be that we have fewer numbers of patients with silent LN (n = 5 of class II) contrary to 26 patients with class I and II of LN in Ishizaki study.

Antibodies against C3 fragments such as C3b have been identified as immunoconglutinins (IK) and reported in patients with SLE at different levels. Rare studies have reported an association between anti-C3b antibodies and LN. In our study, although anti-C3b compared with anti-C1q was less sensitive (34% vs. 43%) for SLE, anti-C1q was more specific (77.5% vs. 75%) for active LN. Indeed, anti-C3b IgG levels inversely correlated with C3 and C4 levels. However, there was no significant difference between the patients with and without anti-C3b antibodies for SLEDAI scores.

In a few numbers of cohort studies, the prevalence of anti-C3b antibodies was reported 30 to 35 percent in SLE patients with kidney involvement [26]. However, our results showed more frequent anti-C3b antibodies in the active LN (45.7%) compared with inactive LN (35%) condition.

These antibodies targeted ‘neoepitopes’ in C3b molecules. Neoepitopes are not available in the native protein and emerge by structural alterations. Some alterations that may lead to the production of neoepitopes occur in processes such as protein activation, proteolytic cleavage, or protein binding [17]. Vasilev et al. demonstrated that these

neoepitopes share among C3, C3b, iC3b, and C3c molecules. Remarkably, they showed autoantibodies also reacted with C4 molecules, indicating that common neoepitopes may exist in all of them. These neoepitopes are formed on the immobilized C3 fragments at the surface of glomeruli and result in kidney damage [26].

This study is accompanied by some limitations. First, these autoantibodies were only detected in sera, while they were not directly evaluated in the pathogenesis of LN. However, it is accepted when they bind deposited complement components or immune complexes, they may develop the inflammation in the site of kidneys. Second, we evaluated limited ethnic group and sample size, thus a multi-racial investigation with a larger-scale population study is necessary to support our data. Third, we did not discriminate different isotypes (or subclasses) among autoantibodies.Finally, our study did not include any follow-up data (serial sampling), especially the assessment of the relationship between LN treatment and autoantibody levels.

Conclusion

We showed that antibodies against dsDNA, C3b, and C1q were more frequent in SLE patients with inactive and active LN. In addition, anti-C1q and anti-dsDNA antibody levels were associated with disease activity and active LN. Notably, a combination of concurrent anti-C1q and anti-dsDNA antibodies results in increased specificity and decrease sensitivity for prediction of active LN. Moreover, our results suggest that the prevalence of these antibodies was associated with severe LN. Taken together, this study supports the view that concurrent anti-C1q and anti-dsDNA (not anti-C3b) antibodies are valuable biomarkers for SLE patients with kidney involvement.

Abbreviations

LN
Lupus Nephritis
SLE
Systemic Lupus Erythematosus
ANAs
Antinuclear antibodies
Anti-dsDNA
anti-double-stranded deoxyribonucleic acid
SLEDAI
SLE Disease Activity Index
IFA
indirect fluorescent assay
HEp-2
Human epithelial type 2
ROC
Receiver operator characteristic

Declarations

Ethics approval and consent to participate

This study was in compliance with the principles of the Declaration of Helsinki and the Ethics Committee for the performance of Human Research of Iran University of Medical Sciences (940330-26574).

Consent for publication

Not applicable.

Availability of data and material

The files of excel (ELISA and other results) and SPSS (statistical analyses) data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors stated no conflict of interest.

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Authors' contributions

All authors have directly participated in the planning, execution, conceptualization, or analysis of this study; and all of them have read and approved the final manuscript. MK acquired, analyzed data, and revised the manuscript, MK, AH, and FS patients selection.MK, RF, MS laboratory experiments. MK, MK, and ND designed the work. MK, FS data analyzed, interpretation, and wrote the original manuscript.

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Figures

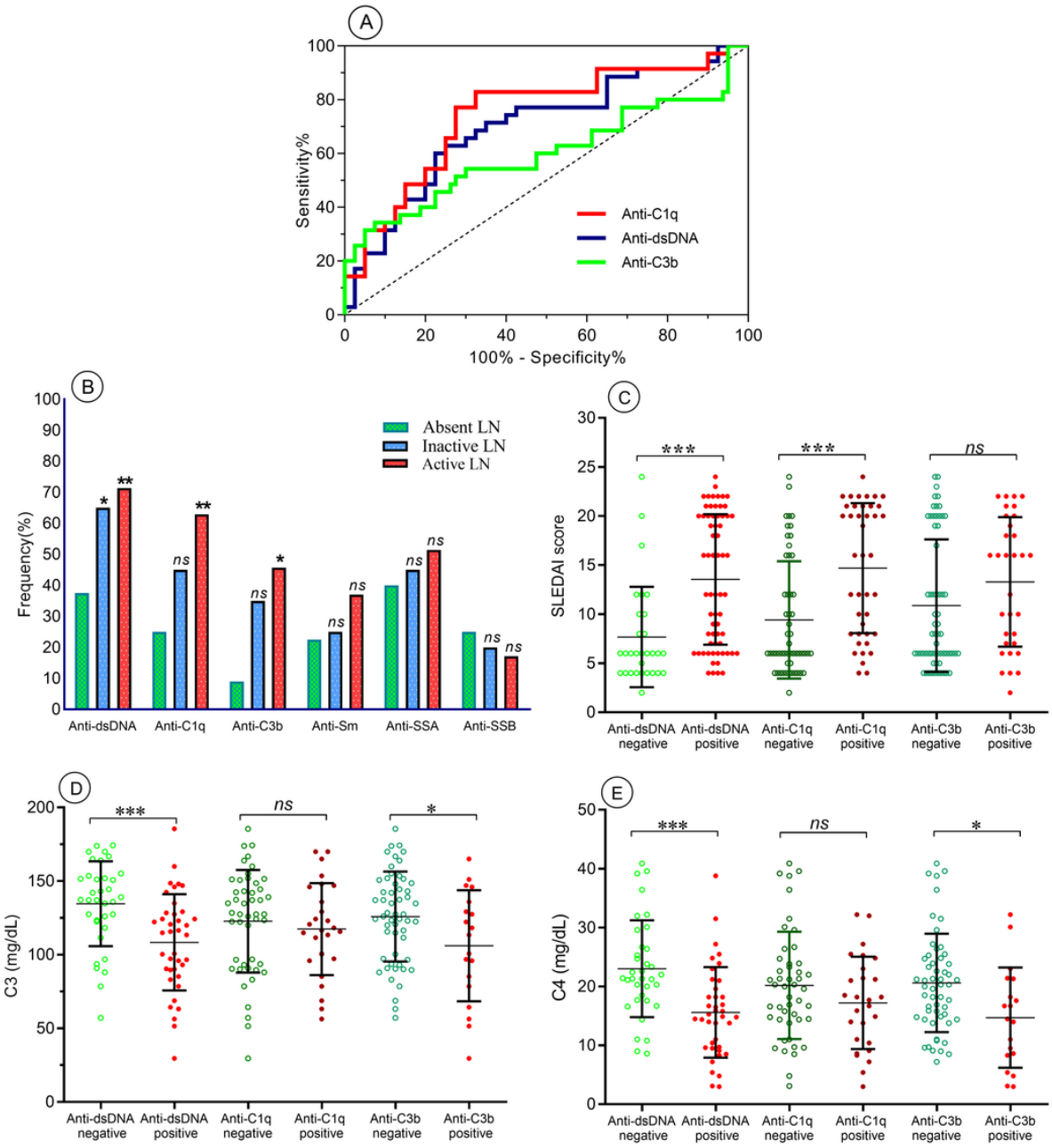

Figure 1

A: ROC curves for anti-dsDNA and anti-C1q antibodies in active LN versus absent LN. B: The prevalence of antibodies against dsDNA, C1q, C3b, Sm, SSA, and SSB in SLE patients with different kidney involvements. The frequency of positive autoantibodies in active and inactive LN groups was compared with absent LN group. C, D, and E: The SLEDAI scores, C3, and C4 levels in anti-dsDNA, anti-C1q, and anti-C3b negative and positive groups of SLE patients. Graphs present as the mean ± SD in each group. *: $p < 0.05$, **: $p < 0.01$, ***: $p<0.001$, and ns: not significant

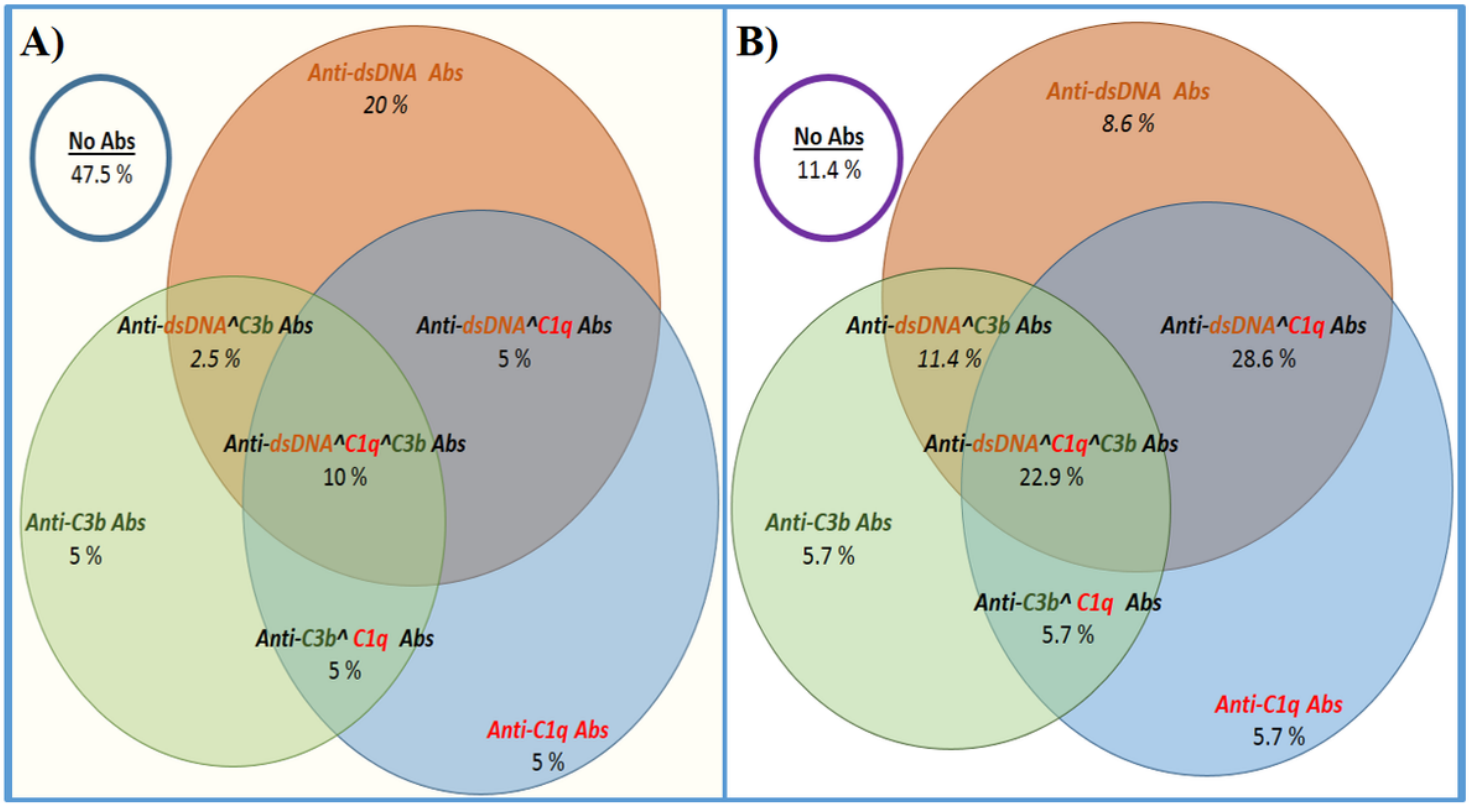

Figure 2

Venn diagrams representing the simultaneous presence of antibodies against dsDNA, C1q, and C3b in SLE patients without kidney involvement (A), and with active lupus nephritis (B).