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ANTI-C1q IN SYSTEMIC LUPUS ERYTHEMATOSUS: RELATIONSHIP WITH CLINICAL MANIFESTATIONS AND DISEASE ACTIVITY

SİSTEMİK LUPUS ERİTEMATOZUSTA ANTİ-C1q: KLİNİK BULGULAR VE HASTALIK AKTİVİTESİ İLE İLİŞKİSİ

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ABSTRACT

Objective: Lupus nephritis (LN) is a detrimental consequence of systemic lupus erythematosus (SLE). The anti-C1q antibody was shown to be related to LN, or global disease activity, in various studies. Our purpose was to determine its prevalence and association with LN or disease activity in Turkish patients with SLE.

Material and Method: We conducted a cross-sectional single-centre study to investigate the clinical and laboratory findings, disease activity, and anti-C1q levels in 150 patients with SLE. The anti-C1q antibody was analyzed using an enzyme-linked immunosorbent assay and compared with 150 healthy-control patients.

Result: Lupus nephritis was present in 72 patients. The frequency of anti-C1q positivity was 17% (26/150) in patients with SLE and 3% (5/150) in control group (p<0.001). Patients with anti-C1q also had anti-Sm, direct Coombs' test, and thrombocytopenia more commonly (p=0.001, p=0.007, p=0.009 respectively). Anti-C1q was positively correlated with proteinuria, haematuria, systemic lupus erythematosus disease activity index (SLEDAI) (p<0.001), anti-dsDNA (p=0.03), and negatively correlated with C3 (p<0.001) and C4 (p=0.015). Patients with active LN had higher anti-C1q (p=0.01) and anti-dsDNA (p<0.001) titres than inactive LN patients, although in multivariate logistic regression analysis, anti-C1q was not significant for LN history. It was significant for SLEDAI severity (p=0.036).

ÖZET

Amaç: Lupus nefriti (LN), sistemik lupus eritematozus'un (SLE) tehlikeli bir sonucudur. Çeşitli çalışmalarda anti-C1q antikorunun LN veya global hastalık aktivitesi ile ilişkili olduğu gösterilmiştir. Bu çalışmada amacımız, Türk SLE'li hastalarda anti-C1q prevalansını, LN veya hastalık aktivitesi ile ilişkisini belirlemekti.

Gereç ve Yöntem: Kesitsel tek merkezli bir çalışma ile 150 SLE'li hastada klinik ve laboratuvar bulguları, hastalık aktivitesi ve anti-C1q düzeyleri değerlendirildi. Anti-C1q antikoru, ELISA (enzyme-linked immunosorbent assay) ile analiz edildi, toplam 150 kişiden oluşan hasta ve sağlıklı kontrol grubu ile karşılaştırıldı.

Bulgular: Yetmiş iki hastada LN'i saptandı. Anti-C1q pozitiflik oranı SLE hastalarında %17 (26/150), kontrol grubunda ise %3 (5/150) idi (p<0,001). Anti-C1q antikoru pozitif olan hastalarda aynı zamanda pozitif anti-Sm antikoru, direkt Coombs testi ve trombositopeni de daha sık görüldü (sırasıyla p=0,001, p=0,007, p=0,009). Anti-C1q antikoru proteinüri, hematüri, sistemik lupus eritematoz hastalık aktivite indeksi (SLEDAI) (p<0,001), anti-dsDNA (p=0,03) ile pozitif, C3 (p<0,001) ve C4 (p=0,015) ile negatif korelasyon gösterdi. Aktif LN'li hastalarda anti-C1q (p=0,01) ve anti-dsDNA (p<0,001) titreleri inaktif LN hastalarına göre daha yüksekti, ancak çok değişkenli lojistik regresyon analizinde anti-C1q LN öyküsü için anlamlı değildi. SLEDAI şiddeti açısından anlamlılık saptandı (p=0,036).

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Conclusion: Our study demonstrated a significant association of anti-C1q with SLE, proteinuria, haematuria, thrombocytopenia, general disease activity, and active LN, but not with inactive renal disease. This is the first study investigating the clinical significance of this antibody in Turkish patients. Further studies are needed to clarify the pathogenesis of lupus nephritis.

Keywords: Anti-C1q, systemic lupus erythematosus, lupus nephritis, systemic lupus erythematosus disease activity index

Sonuç: Çalışmamız anti-C1q antikorunun SLE, proteinüri, hematüri, trombositopeni, genel hastalık aktivitesi ve aktif LN ile anlamlı bir ilişkisi olduğunu, ancak inaktif böbrek hastalığı ile ilişkili olmadığını kanıtladı. Çalışmamız, bu antikorun Türk SLE'li hastalarda klinik önemini araştıran ilk araştırmadır. Lupus nefritinin patogenezini açıklığa kavuşturmak için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Anti-C1q, lupus nefriti, sistemik lupus eritematozus, sistemik lupus eritematozus hastalık aktivite indeksi

INTRODUCTION

Systemic lupus erythematosus (SLE) is characterized by various autoantibody production processes that contribute to inflammatory damage across various organ systems. Lupus nephritis (LN) is a frequent and severe condition, often indicating a worse prognosis (1). Prompt diagnosis and treatment are essential for improving LN outcomes and survival in patients with SLE (2). However, the gradual onset and unpredictable course of LN present challenges in diagnosis and monitoring. A biomarker capable of predicting LN flares before noticeable changes in proteinuria, urine sediment, or decline in kidney function that can be routinely monitored during patient visits would be invaluable for initiating treatment early and preventing significant renal damage (3).

Increased levels of anti-double-stranded DNA (anti-dsDNA) antibodies and reduced complement levels were linked to active SLE. However, their lack of specificity for renal flares has led to a search for other antibodies (4, 5). Complement activation is crucial in the development of both SLE and LN. C1q, the initial component of the classical complement pathway, participates in the removal of immune complexes formed during apoptosis from tissues (6). Although genetic C1q deficiency is linked to SLE, in most SLE patients, C1q deficiency is a secondary event associated with anti-C1q antibodies. These antibodies can impede the neutralisation of immune complexes with C1q, leading to their deposition, complement activation, and subsequent inflammation (7).

Various studies have investigated the link between anti-C1q antibodies and LN or global activity in SLE. Some of them suggested that anti-C1q antibodies are superior markers for identifying renal flares (8, 9). In contrast, others have argued that combining anti-C1q antibodies with other antibodies provides better predictive value than using anti-C1q antibodies alone (10-12). Most of these studies have stated that the absence of this antibody is related to a lower possibility of LN flares (10). A multinational study stated that anti-C1q levels were parallel to activity levels measured by the modified Safety of Estrogen in Lupus: National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) and the Systemic Lupus International Collaborating Clinics (SLICC) Renal Activity Score (RAS) (7). However, researchers have argued that anti-C1q antibodies are related to overall disease activity, not necessarily nephritis (13). Two meta-analyses aimed to resolve the conflicting findings regarding anti-C1q antibodies. Yin et al. suggested that anti-C1q antibodies could be an informative tool for forecasting LN and measuring active nephritis, whereas Eggleton et al. did not find sufficient evidence to support this association (14, 15).

In the current study, our primary goal was to assess the importance of the anti-C1q antibody in Turkish SLE patients and to analyse its connexion with LN and disease activity.

MATERIAL AND METHODS

Patients

We conducted a controlled cross-sectional study at our university hospital rheumatology outpatient clinic from January 2016 to January 2017. Our clinic serves as a tertiary referral centre for rheumatology.

One hundred and fifty consecutive SLE patients were enrolled. All patients were diagnosed with SLE, either by fulfilling at least four of the American College of Rheumatology (ACR) revised diagnostic criteria for SLE or 4 of the SLICC 2012 diagnostic criteria (16, 17).

During routine outpatient follow-up visits, we recorded demographics including age and gender. In addition, we measured levels of anti-C1q, anti-dsDNA, anti-Smith (anti-Sm), complement components C3 and C4, performed a Coomb's test, obtained a complete blood count (CBC), and analysed urine for red blood cell (RBC) casts and 24-h urine protein levels. Clinical manifestations and treatments received by the patients up to the study period were also evaluated to define their general characteristics (Table 1).

Disease activity was defined according to SLEDAI 2000 (SLEDAI-2K) criteria (18). A SLEDAI score of 0-3 was classified as inactive disease, 4-8 as mild, 9-12 as moderate,

Table 1: Clinical, laboratory findings, and treatments of SLE patients until the time of study n (%)

Photosensitivity	94 (62.7)	Anti-dsDNA (+)	36 (24)
Malar rash	71 (47.3)	Anti-Histone (+)	33 (22)
Discoid rash	13 (8.7)	Anti-Sm (+)	14 (9.3)
Oral ulcer	28 (18.7)	Anti-Sm/RNP	44 (29.3)
Alopecia	41 (27.3)	Anti-SSA (+)	57 (38)
Arthritis	101 (67.3)	Anti-SSB (+)	21 (14)
Pleuritis	25 (16.7)	Anti-Nucleosome (+)	57 (38)
Pericarditis	18 (12)	Anti-Rib P Protein (+)	21 (14)
Seizure	5 (3.3)	LAC[b]	17 (11.3)
Lupus nephritis	72 (48)	ACLA[c] IgG (+)	5 (3.3)
Proteinuria	16 (10.7)	ACLA IgM (+)	2 (1.3)
Haematuria	14 (9.3)	Steroid	148 (98.7)
Leukopoenia	24 (16)	Hydroxychloroquine	150 (100)
Lymphopenia	35 (23.3)	Azathioprine	108 (72)
Haemolytic anaemia	12 (8)	Mycophenolate mofetil	54 (36)
Thrombocytopenia	6 (4)	Cyclophosphamide	44 (29.3)
APS[a] history	24 (16)	Rituximab	15 (10)
Coomb's test (+)	25 (16.7)	Intravenous immune globulin	3 (2)
Low C3/C4	46 (30.7)	Plasmapheresis	1 (0.7)

[a]: Antiphospholipid syndrome, [b]: Lupus anticoagulant, [c]: Anti-cardiolipin antibody

and ≥12 as severe activity. Patients with moderate to severe disease activity (SLEDAI score ≥9) were considered to have active disease. Urine protein excretion ≥500 mg/day or the presence of ≥5 RBC casts per high-power field (HPF) was interpreted as active nephritis. Renal biopsies were assessed based on the revised International Society of Nephrology and Renal Pathology Society (ISN/RPS) classification (19).

The disease control (DC) group comprised 101 consecutive patients followed at the same clinic. The healthy control (HC) group included 49 individuals with no history of chronic diseases.

For anti-C1q antibody measurement, sera were collected and frozen as 100 µL samples at minus 80°C until analysis.

The Kocaeli University Ethics Committee approved the research protocol, and all participants provided written consent (Date: 11.12.2015, No: KAEK/2015/133-16/19).

Anti-C1q IgG antibodies

Enzyme-linked immunosorbent assay (ELISA) kits (ORG 549, Orgentec Diagnostika GmbH, Mainz, Germany) were utilised for anti-C1q antibody detection. Initially, sera were diluted by 1/100, introduced into the wells, and incubated for 30 min at room temperature (RT). After three washes with the wash solution, 100 µL of enzyme conjugate was introduced into the wells and incubated again at RT.

Following a 15-min incubation, each well was washed three times, and 100 µL of trimethyl benzene solution was introduced, followed by further incubation for 15 min at RT. Finally, 100 µL of stop solution was applied to the wells, and optical density was assessed at 450 nm. The results were used to determine the concentrations based on a predefined conversion method. Ten U/mL was set as the cut-off value for anti-C1q, with values ≥10 U/mL interpreted as positive per manufacturer.

Other tests

The anti-nuclear antibody (ANA) test was conducted with an indirect immunofluorescence assay (Euroimmun, Luebeck, Germany), with titres of 1:160 considered as the cut-off value. Anti-extractable nuclear antigen (ENA) antibodies were detected with an immunoblotting assay (Euroimmun, Luebeck, Germany). Anti-dsDNA antibodies were measured using ELISA (Euroimmun, Luebeck, Germany). Serum C3 and C4 levels were measured using Beckman Coulter reagents on the AU5800 analyser (Brea, California, USA). CBC measurements were performed using a Beckman Coulter DxH800 Hematology Analyzer (Brea, California, USA). The Coombs test was conducted using the Beckman Coulter Across Auto System Octom (Brea, California, USA). RBC casts were detected using a Beckman Coulter iQ200 Sprint urine microscopy system (Brea, California, USA). Twenty-four-hour urine protein levels were measured spectrophotometrically using Beckman Coulter reagents on the AU5800 Analyser (Brea, California, USA).

Statistical analysis

SPSS© 25.0 (IBM Statistical Package for Social Sciences, Corp., Armonk, NY, USA) and R© programmes were utilised for analysis. Gender, clinical and laboratory findings, and treatments patients received until the time of study were expressed as frequencies (the number of cases) and relative frequencies (percentages). Age, anti-C1q and an-

ti-dsDNA titres, proteinuria, haematuria, white blood cell (WBC), RBC, platelet (PLT) counts, and C3 and C4 levels were reported as mean values ± standard deviation (SD) in cases of normal distribution. Kolmogorov-Smirnov test was utilised to cheque distribution.

The chi-square test or Fisher exact test was used to evaluate clinical and laboratory findings based on anti-C1q antibody status. The difference in anti-C1q titres between patients and controls was analysed using the Mann–Whitney U test. Likewise, the differences in anti-dsDNA titres, proteinuria, haematuria, WBC, RBC, PLT counts, and C3 and C4 levels between patients based on anti-C1q antibody status were analysed with t-test or Mann-Whitney U test.

To evaluate the difference in anti-C1q and anti-dsDNA titres, as well as C3 and C4 levels between patients with and without LN based on disease activity, t-tests or Mann-Whitney U tests were used, based on the normality of the data.

For comparison of all four of these groups separately, ANOVA or Kruskal-Wallis test with Bonferroni analysis was utilised. Correlations between anti-C1q, anti-dsDNA, C3, and C4 were analysed using the Spearman correlation test. Furthermore, logistic regression analysis was performed to estimate the impact of anti-C1q, anti-dsDNA, C3, and C4 on LN and disease activity. A p-value less than 0.05 was defined as statistically significant.

RESULTS

SLE and control group characteristics

One hundred and fifty consecutive patients with SLE (138 female, 12 male) were enrolled. Patients' ages ranged between 19 and 82 years with a mean of 46±12.8. The average duration of disease was 74.3±51.4 months. Arthritis was the most common symptom (67.3%), followed by photosensitivity (62.7%) and malar rash (47.3%). Twenty-four patients had anti-phospholipid syndrome.

Of the 72 patients with a history of LN, only three patients did not undergo a renal biopsy. Among those who underwent biopsies, one patient's result was non-diagnostic. The pathological diagnoses were as follows: class II LN was present in 24 patients, class III in eight, class IV in 26, and class V in 10. The glomerular filtration rate (GFR) was below 60 ml/min in 12 patients.

In patients with LN, 26 had mild, four had moderate, and four had severe active disease. Patients without LN had less active disease; 13 had mild, two had moderate, and two had severe active disease. Table 1 presents the clinical and serological information, and the treatments received by SLE patients up to the time of the study.

There were 101 patients in the DC group, as follows: rheumatoid arthritis (n=85), Sjögren syndrome (n=8), adult-onset Still's disease (n=3), systemic sclerosis (n=2), and psoriatic arthritis (n=3), all meeting their respective diagnostic criteria. The HC group comprised 49 individuals without any chronic diseases. Age and gender were similar between the SLE and control groups, as detailed in Table 2.

The prevalence and titres of anti-C1q antibodies

The prevalence of anti-C1q antibodies in SLE patients was 17% (26/150) and was significantly higher than that in DC (3/101) and HC (2/49) subjects (p<0.001 calculated both combined and separately for each control group).

Additionally, compared with both control groups, the titres of anti-C1q antibodies were considerably greater in SLE patients (p<0.001) (Table 2).

Anti-C1q antibodies and clinical, laboratory findings

Patients who had anti-C1q antibodies had a greater amount of proteinuria (p=0.047) than those without this antibody, despite a similar prevalence of LN between the two groups. Additionally, these patients had more anti-dsDNA (p=0.016 for prevalence and p=0.014 for titre) and lower C3 levels (p=0.009), despite similar prevalence of low C3 across groups (p=0.131). Conversely, both the prevalence of low C4 (p=0.017) and lower C4 levels (p=0.001) were significantly higher in patients who had anti-C1q antibodies. Furthermore, these patients also had more anti-Sm antibodies (p=0.001) and Coombs' tests (p=0.007). Although thrombocytopenia was prevalent among these patients (p=0.009), platelet numbers were similar (p=0.779). Only a few patients with extremely low platelet counts could be the reason for this. Table 3 summarises clinical and laboratory features of SLE patients based on anti-C1q antibody status.

Correlations with other parameters

We observed a positive correlation between anti-C1q and SLEDAI (r=0.378, p<0.001), anti-dsDNA (r=0.178, p=0.03), proteinuria (r=0.286, p<0.001), and RBC casts (r=0.438, p<0.001). Conversely, we also found a negative correlation between this antibody and C3 (r=-0.322, p<0.001) and C4 (r=-0.198, p=0.015) (Figure 1). We did not find any correlation with WBC, lymphocyte, or PLT counts.

Table 2: Demographics and anti-C1q antibody levels in each group

	SLE group (N=150)	Controls (N=150)	AntiC1q
Female/Male	138/12	132/18	p=0.248
Age (years)	46±12.8 (19-82)	45.5±14.5 (17-76)	p=0.943
Anti-C1q (+)[b]	26 (17)	5 (3)	p<0.001*
Anti-C1q titres[c]	8.42±16.02	3.47±3.97	p<0.001*

[a]: Mean±SD (Range), [b]: n (%), [c]: Mean±SD, *:p<0.05

Table 3: Clinical and laboratory findings associated with anti-C1q antibody at the time of the study

	n (%)	n (%)	p	Odds ratio (CI)
LN history[a]	14 (53.8)	58 (46.7)	0.512	1.328 (0.5-3.1)
Proteinuria[b]	4 (15.3)	12 (9.6)	0.482	1.697 (0.5-5.7)
Haematuria[b]	5 (19.2)	9 (7.2)	0.069	3.042 (0.9-9.9)
Leukopoenia[b]	6 (23)	18 (14.5)	0.375	1.767 (0.6-4.9)
Lymphocytopenia[a]	8 (30.7)	27 (21.7)	0.324	1.597 (0.6-4.1)
Thrombocytopenia[b]	4 (153)	2 (1.6)	0.009*	11.09 (1.9-64.2)
Anti-Sm (+)[b]	7 (26.9)	7 (5.6)	0.003*	6.158 (1.9-19.5)
Coombs' test (+)[a]	9 (34.6)	16 (12.9)	0.007*	3.574 (1.4-9.4)
Anti-dsDNA (+)[a]	11 (42.3)	25 (20.1)	0.016*	2.904 (1.2-7.1)
Low C3[a]	8 (30.7)	22 (17.7)	0.131	2.061 (0.8-5.3)
Low C4[a]	13 (50)	30 (24.1)	0.017*	0.176 (0.0-1.4)
	Mean±SD	**Mean±SD**		
Proteinuria (mg/day)[d]	603.15±1014.83	2.94.77±774.46	0.047*	
Haematuria (RBC/HPF)[d]	4.31±8.21	2.23±4.11	0.484	
WBC (1000/µL)[d]	6081.54±3203.58	6666.08±2939.90	0.210	
Lymphocytes (1000/µL)[d]	1659.38±1126.85	1702.67±881.41	0.384	
Platelets (1000/µL)[c]	235984.6±107637.15	241118.9±79325.19	0.779	
Anti-dsDNA (IU/ml)[c]	230.08±322.79	77.32±216.33	0.014*	
C3 (mg/dl)[c]	87.95±36.26	108.86±27.60	0.009*	
C4 (mg/dl)[d]	26±13.57	22.09±16.06	0.001*	

[a]: Chi-square test, [b]: Fischer exact test, [c]: t-test, [d]: Mann-Whitney U test is used for analysis, *:p<0.05, CI: Confidence interval

Anti-C1q antibodies and the general disease activity

We examined the effect of anti-C1q antibodies on the disease activity. Patients who had anti-C1q antibodies exhibited higher SLEDAI scores (p=0.009). Subsequently, we stratified SLEDAI scores based on mild or moderate-severe disease activity (SLEDAI≥ 9). Patients who had anti-C1q antibodies demonstrated more active disease (p=0.004), and those with active disease displayed higher anti-C1q antibody titres (p=0.001) (Figure 2).

Moreover, multivariate logistic regression analysis highlighted the statistical significance of anti-C1q (p=0.036) and anti-dsDNA antibody (p=0.002) effects on SLEDAI severity scores. Patients who had anti-C1q antibodies had 4.5 times and those who had anti-dsDNA antibodies had 13 times active disease.

Anti-C1q antibodies and renal disease activity

Patients with LN had more disease activity (SLEDAI≥ 9) than patients without LN (p=0.003 for prevalence and p=0.002 for SLEDAI scores) (Figure 3).

Initially, we conducted separate analyses for patients with and without LN to identify any differences in characteristics or antibody profiles between the two groups. LN patients with active disease had greater anti-C1q and anti-dsDNA antibody titres than patients with inactive LN (p=0.010, p<0.001, respectively).

Among patients without LN, anti-C1q antibody titres were similar between patients regardless of activity status. However, active patients had greater anti-dsDNA antibody titres (p=0.001) and significant hypocomplementemia (p=0.019 for C3, p=0.044 for C4) (Table 4).

Subsequently, we categorised patients based on LN history and activity into four groups and analysed them using Bonferroni analysis. Patients who had active LN exhibited higher anti-C1q and anti-dsDNA antibody titres compared to patients with inactive LN and those with no LN history (p=0.013, p<0.001, respectively). In addition, they had lower C3 levels compared with both inactive patients regardless of LN status (p=0.002), and their C4 levels were lower than those of inactive LN patients (p=0.004) (Table 5).

In multivariate logistic regression analysis, we were not able to prove any effect of anti-C1q, anti-dsDNA, C3, and C4 on LN history.

DISCUSSION

LN still significantly impacts morbidity and survival in patients with SLE (20). It is imperative to identify markers for LN to forecast renal involvement, reflect clinical and pathological disease activity, monitor relapse, and guide therapeutic options (21). In SLE, organ damage results from the interplay of autoantibody production, immune complex deposition, and immune tolerance dysfunction affecting multiple organs (22). The complement system is crucial for clearing immune complexes and autoantigens produced during cell apoptosis, thereby protecting against autoimmune-mediated tissue and organ damage (23). Antibodies to C1q are among the extensively stud-

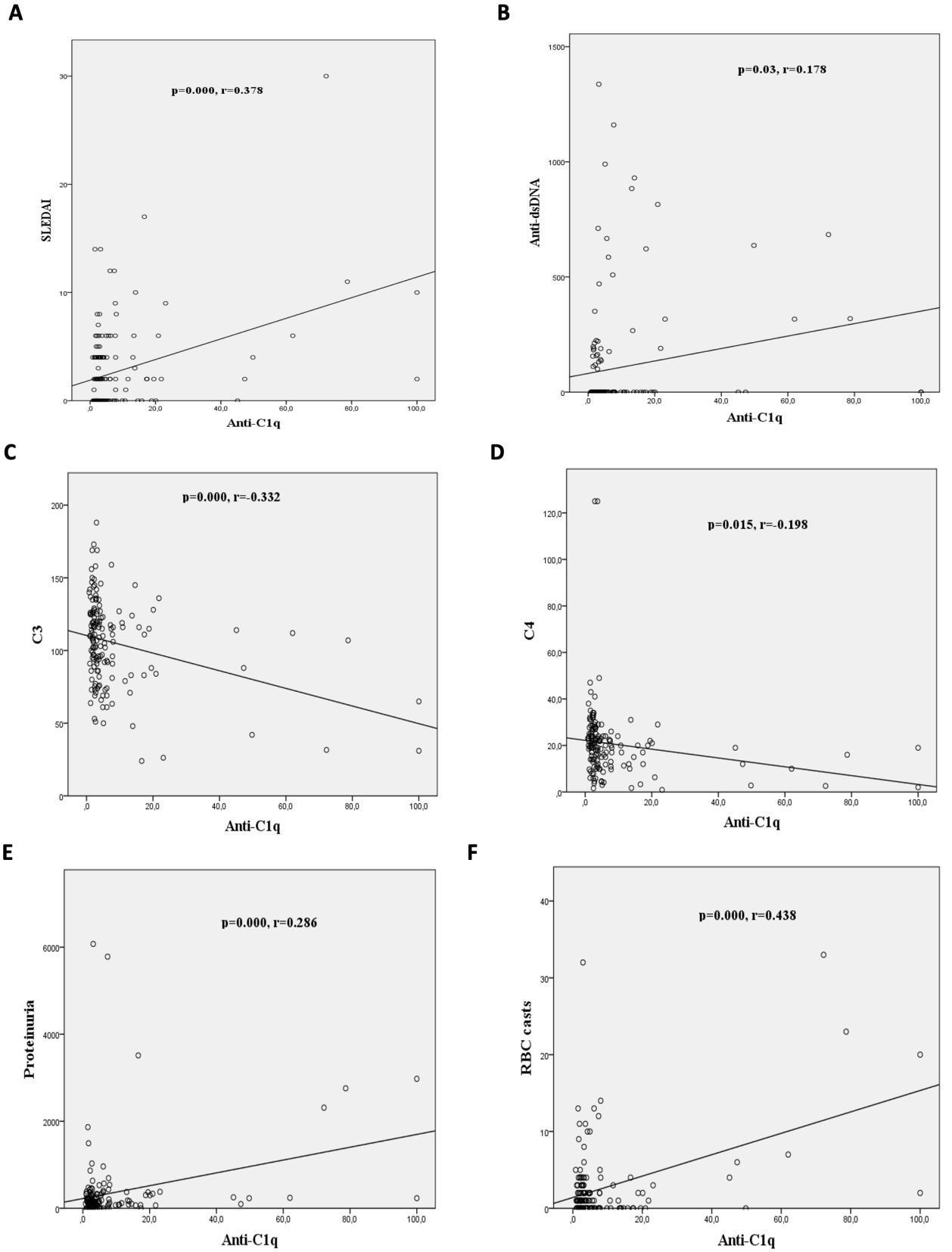

Figure 1: Correlations between anti-C1q antibody titres and A. systemic lupus erythematosus disease activity index (SLEDAI); B. anti-dsDNA; C C3; D C4; E proteinuria (mg/dl); and F Red blood cell casts/High power field (RBC casts/

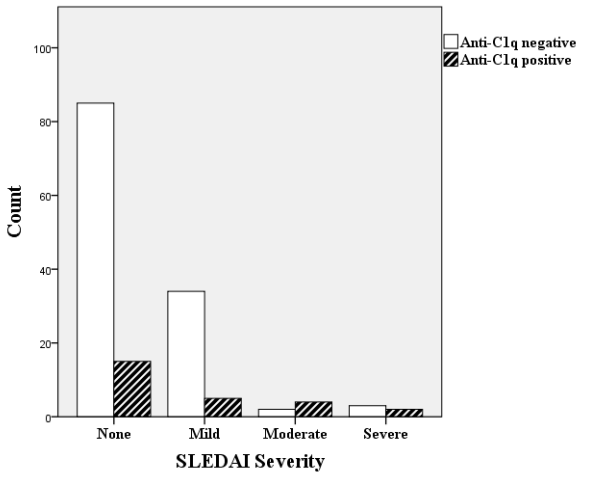

Figure 2: Anti-C1q prevalence in relation to systemic lupus erythematosus disease activity index (SLEDAI) severity. Bars indicate numbers of patients with and without anti-C1q antibody. SLEDAI severity scores; none: 0-3 points, mild: 4-8 points, moderate: 9-12 points, severe ≥12 points. Patients with anti-C1q antibody had more active disease (p=0.004).

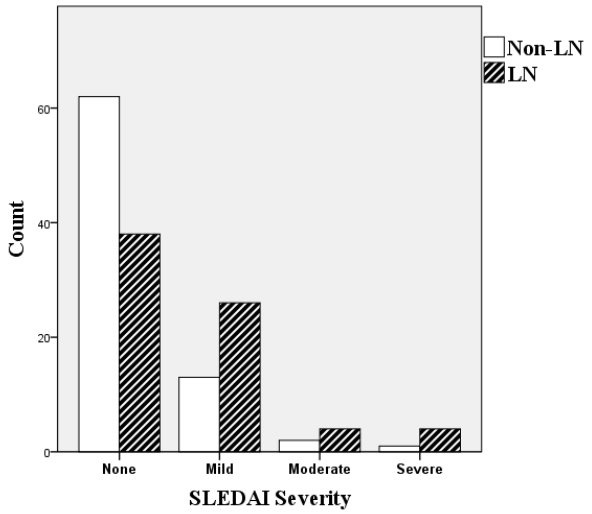

Figure 3: Lupus history in relation to systemic lupus erythematosus disease activity index (SLEDAI) severity. Bars indicate the number of patients with lupus nephritis (LN), and those without LN history (Non-LN). SLEDAI severity scores; none: 0-3 points, mild: 4-8 points, moderate: 9-12 points, severe ≥12 points. Patients with renal disease history had more active disease (p=0.003).

ied serological markers in SLE, representing a key focus in understanding these mechanisms (14, 15).

Ethnicity plays a significant role in the clinical manifestations, disease activity, organ damage, and treatment response observed in LN (24). To address these complexities, we conducted a single-centre, cross-sectional study investigating the presence of anti-C1q antibodies in Turkish patients with LN. To our knowledge, this research represents the first examination of anti-C1q antibodies in this population. Examining 150 SLE patients and 150 DC and HC subjects in total, we proved a link between anti-C1q antibodies and both the diagnosis and global disease activity of SLE. The single-centre nature of our study enabled a uniform analysis of patient data and laboratory findings. However, the prevalence of this antibody in our study (17%) was less than that reported previously, in the range of 29-60% (25).

Studies have shown that anti-C1q antibodies vanish following immunosuppressive treatment, becoming undetectable by the third month and remaining undetectable during the first year of follow-up (26). Given that most of our patients had been diagnosed and treated earlier and were in remission (average disease duration of 74.3±51.4 months, with only 7% of patients having active disease), this could explain the lower prevalence in our study.

Comparing results from different studies is challenging because of the lack of a uniform assay for anti-C1q antibody ELISA. Assays vary in terms of assay conditions and the antigen used. In our study, we used native human C1q as the antigen. A study by Jaekel et al. reported that the specificity of this assay for SLE was 89% and that for LN was 84%, but the sensitivity for SLE was 34% and that for LN was 64% (27). The high specificity but low sensitivity of our assay could also contribute to the lower prevalence of this antibody observed in our study.

In this study, patients who had anti-C1q antibodies more commonly had thrombocytopenia. Various studies have investigated the link between anti-C1q antibodies and haematologic findings. For example, a previous study reported an association between leukopoenia and this antibody (13). Armstrong et al. also emphasised the importance of anti-C1q antibodies in LN and the haematological findings of SLE (28). However, another study found no difference in findings (29). The clinical importance of thrombocytopenia in our study requires further verification.

Anti-C1q antibodies were correlated with anti-dsDNA, C3 and C4 levels, and SLEDAI, confirming its function as a valuable indicator of disease activity, as previously mentioned (11, 25). Patients with anti-C1q antibodies exhibited more active disease. In multivariate logistic regression analysis, these patients also had 4.5 times higher odds of having active disease, whereas those with anti-dsDNA antibodies had 13 times higher odds.

In our study, we observed a relationship between anti-C1q antibodies and proteinuria, haematuria, and active LN, but not with inactive LN. Although the prevalence of anti-C1q antibodies was not influenced by the history of LN, there

Table 4: Comparison of anti-C1q, anti-dsDNA antibody, C3, C4 titres according to disease activity in patients with and without lupus nephritis (LN) separately

	Active LN (n=8)	Inactive LN (n=64)	p	Active non-LN (n=3)	Inactive non-LN (n=75)	p
Anti-C1q	36.27±40.19	7.37±14.45	0.010*	13.40±9.96	6.14±9.61	0.096
Anti-dsDNA	421.13±398.43	43.42±134.71	<0.001*	861.33±513.46	91.17±211.44	0.001*
C3[a]	74.61±47.48	107.13±25.81	0.096	67.07±53.04	108.42±28.42	0.019*
C4	15.11±1482	22.40±16.27	0.065	7.30±10.40	20.21±14.53	0.044*

[a]: Only C3 is analysed with t-test, all other variables were analysed with Mann-Whitney U test, *:p<0.05

Table 5: Comparison of anti-C1q, anti-dsDNA antibody, C3, C4 titres according to disease activity and lupus nephritis (LN) history in four different groups separately

	Active LN (n= 8) (1)	Inactive LN (n=64) (2)	Active non-LN (n=3) (3)	Inactive non-LN (n=75) (4)	p	Bonferroni
Anti-C1q (U/ml)	36.27±40.19	7.37±14.45	13.40±9.96	6.14±9.61	0.013*	2<1, 4<1
Anti-dsDNA (IU/ml)	421.13±398.43	43.42±134.71	861.33±513.46	91.17±211.44	<0.001*	2<1, 2<3, 4<1, 4<3
C3 (mg/dl)[a]	74.61±47.48	107.13±25.81	67.07±53.04	108.42±28.42	0.002*	1<2, 1<4
C4 (mg/dl)	15.11±1482	22.40±16.27	7.30±10.40	20.21±14.53	0.004*	1<2, 3<2

[a]: Only C3 is analysed with ANOVA, all the other variables were analysed with Kruskal-Wallis test. Bonferroni analysis was performed, *:p<0.05.

was an association with the level of proteinuria, in line with the findings of a previous investigation by Petri et al (7). We also observed positive correlations between anti-C1q antibodies and proteinuria and haematuria.

When compared separately, patients who had active LN had higher titres of anti-C1q in comparison to patients with inactive LN and inactive non-LN. Marto et al. reported similar findings in their study: the frequency of anti-C1q antibodies was not different in patients with LN based on disease activity, but active patients had higher antibody titres (30). We were not able to show any effect of anti-C1q, anti-dsDNA, C3, and C4 in multivariate logistic regression for LN history. Our patients were more likely to represent rheumatology outpatient clinics. Most of the studies showing a relationship between anti-C1q antibodies and LN were from nephrology departments with more patients with active renal disease. As in previous studies, our results suggest that anti-C1q antibodies are related to general disease activity but not specifically to LN (29, 30).

In the meta-analysis by Eggleton et al., 31 studies were analysed to detect the accuracy of the anti-C1q antibody among patients with SLE. The authors concluded that, for distinguishing between those with and without a history of LN and the activity of patients with LN, the choice of anti-C1q antibodies as a singular diagnostic marker was not found to be useful. Post-test probabilities after a positive test were generally too low, and after a negative test, they were generally too high to be certain about the condition (15). Our findings are similar to these results.

In a recent systematic review on prognostic factors in LN and an overview of systematic reviews on the diagnostic accuracy of LN biomarkers, both sets of authors concluded that definitive biomarkers for these purposes were still lacking and further studies were needed (31, 32).

The limitations of our study include the limited number of patients with LN and the lack of repeated measures or information on disease flares because of its cross-sectional nature. In addition, being a single-centre study, the findings may not be representative of the general population of Turkish SLE patients, which could limit the generalizability of our results. Future studies could benefit from enrolling more patients with active disease, measuring anti-C1q titres before and after treatment, and obtaining repeated measures over time to better understand anti-C1q's role in diagnosing and monitoring patients with SLE.

CONCLUSION

In summary, anti-C1q antibodies are linked to SLE and overall disease activity, including active LN. Although we did not find an association with renal disease history, proteinuria was significant, and patients with active disease exhibited higher antibody titres.

Ethics Committee Approval: The study was approved by the Kocaeli University ethical committee (Date: 11.12.2015, No: 19).

Informed Consent: Consent was obtained from all participants.

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