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Serological Evaluation of Systemic Lupus Erythematosus: Association with Disease Severity in Iraqi Patients

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Abstract

The diagnosis, management and treatment of systemic lupus erythematosus (SLE) in clinical settings often involve serological measures of autoantibodies, specifically the anti-double stranded DNA and generally the anti-nuclear autoantibodies (ANA). It remains to be shown, however, whether these serological measures correspond with the severity classification of SLE, particularly among Iraqi patients. Here, we investigated the relationship between serological measurements of autoantibodies and complement proteins with severity classification of SLE patients. Sixty patients clinically diagnosed with SLE, along with 60 age-matched non-SLE individuals (control) were recruited in the study. Serum levels of ANA, anti-dsDNA, complement C3 and C4 were measured. The SLE patients had significantly higher mean values of ANA, anti-dsDNA, with significantly lower levels of C3 and C4 compared to those of the control (non-SLE). With respect to their SLE disease severity classification, while levels of ANA significantly increased with severity of disease (mild, 7.99±0.65 IU/mL; 9.83±0.93 IU/mL; severe, 13.70±1.60 IU/mL; $p = 0.004$), levels of anti-dsDNA despite increasing (mild, 33.38±2.18 IU/mL; moderate, 39.32±2.28 IU/mL; severe, 42.84±4.80 IU/mL), were not statistically significant ($p = 0.101$). ($p < 0.05$). Conversely, levels of C3 (mild, 1.19±0.05 g/L; moderate, 0.98±0.11 g/L; severe, 0.72±0.17 g/L; $p = 0.009$) and C4 (mild, 0.32±0.02 g/L, moderate, 0.29±0.03 g/L; severe, 0.16±0.03 g/L; $p = 0.002$) significantly decreased with disease severity ($p < 0.05$). Our findings show that although ANA and anti-dsDNA autoantibodies may be important in the diagnosis of SLE, their use in predicting the severity of the disease varies considerably, while also highlighting, the significance of complement components C3 and C4 in monitoring and predicting the severity of the disease in addition to their role in the diagnosis of SLE.

التقييم المصلي لمرض الذئبة الحمامية الجهازية: الارتباط مع شدة المرض لدى المرضى العراقيين

فاطمة عبد الحسين كاظم، ستار جبار راهي الغرايتي، تماضر صباح مهدي الحسناوي، رياض ضيهوود الزبيدي

الملخص

غالبًا ما يتضمن تشخيص وإدارة وعلاج الذئبة الحمامية الجهازية SLE في الحالات السريرية قياسات مصلية للأجسام المضادة الذاتية، وتحديدًا الحمض النووي المضاد المزدوج والأجسام المضادة الذاتية المضادة للنواة (ANA) بشكل عام. ومع ذلك، يبقى أن نوضح ما إذا كانت هذه القياسات المصلية تتوافق مع تصنيف شدة مرض الذئبة الحمراء، خاصة بين المرضى العراقيين. هنا، قمنا بدراسة العلاقة بين القياسات المصلية للأجسام المضادة الذاتية والبروتينات التكميلية مع تصنيف شدة مرضى الذئبة الحمراء. تم تجنيد ستين مريضًا تم تشخيص إصابتهم بمرض الذئبة الحمراء سريريًا، إلى جانب 60 شخصًا غير مصابين بمرض الذئبة الحمراء (الضابطة) في الدراسة. تم قياس مستويات مصلANA ، ومضاداتdsDNA ، ومكمل C3 و .C4كان لدى مرضى الذئبة الحمراء (SLE) قيم متوسطة أعلى بكثير للـANA ، والمضادات للـdsDNA ، مع مستويات أقل بكثير من C3 و C4مقارنة بتلك الموجودة في المجموعة الضابطة (غير المصابين بمرض الذئبة الحمراء). فيما يتعلق بتصنيف شدة مرض الذئبة الحمراء، في حين أن مستويات ANA زادت بشكل ملحوظ مع شدة المرض (خفيف , ;7.99±0.65 IU/mL 9.83±0.93 IU/mL , 13.70±1.6 IU/mL شديد **(**p = 0.004)مستويات مضادات dsDNA على الرغم من الزيادة (خفيف، 33.38±2.18 IU/mL ؛ معتدل، 39.32±2.28 IU/mL ؛ شديد 42.84±4.80 IU/mL)لم تكن ذات دلالة إحصائية(p <0.05 (p = 0.101)) على العكس من ذلك، مستويات C3 (خفيف، 1.19±0.05 جم/لتر؛ معتدل، 0.98±0.11 جم / لتر، شديد، 0.72 + 0.17 جم / لتر)، (p = 0.009) ,C4 (معتدل، 0.32 + 0.02 جم / لتر، معتدل، 0.29 ± 0.03 جم / لتر، شديد، 0.16 0.03 جم / لتر)، **(**p 0.002) بشكل ملحوظ انخفض مع شدة المرض (P <0.05) تظهر النتائج التي توصلنا إليها أنه على الرغم من أن الأجسام المضادة ANA والأجسام المضادة لـ dsDNA قد تكون مهمة في تشخيص مرض الذئبة الحمراء، إلا أن استخدامها في التنبؤ بخطورة المرض يختلف بشكل كبير، مع تسليط الضوء أيضًا على أهمية المكونات التكميلية C3 و C4في مراقبة وتوقع شدة المرض. للمرض بالإضافة إلى دورها في تشخيص مرض الذئبة الحمراء

1. Introduction

Systemic lupus erythematosus (SLE) is a multi-system inflammatory autoimmune disease that affects several organ systems to differing degrees of severity. It has a wide variety of clinical symptoms. Some individuals only experience arthralgias and rashes, whereas others have serious multi-organ involvement, such as vasculitis or nephritis (Connelly and Morand, 2021). SLE is characterized by inefficient apoptotic clearance, deregulation of the immune system, complement activation, immune complexes, and tissue inflammation. Although the exact a etiology of this illness is still unknown, research suggests that a combination of genetic and environmental factors trigger immunological responses, which in turn cause B cells to produce autoantibodies and dysregulate cytokines, both of which harm tissue and organs. Antibodies against nuclear and cytoplasmic antigens are indicative of SLE (Agmon-Levin et al., 2012). Symptoms of SLE can affect one or more organ systems, range in severity, and fluctuate over time and this occasionally makes diagnosing this illness challenging (Ding et al., 2023). Common signs of flare-ups in systemic lupus include skin rashes such as the malar "butterfly rash," arthritis, pleurisy, serositis, alopecia, and lupus nephritis. Unfortunately, patients and doctors alike may become frustrated by the fact that treatment response varies and can be difficult to predict (Lazar and Kahlenberg, 2023). As a result, the clinical signs of SLE vary widely, ranging from minor skin involvement to serious organ damage such kidney failure, pulmonary hypertension, and heart failure, all of which are non-specific (Moutsopoulos and Zampeli, 2021).

Individualized research on SLE patients is complicated by the heterogeneity of SLE severity since it might mediate, confuse, or modify correlations with outcomes; hence, it is best to stratify or account for this severity under different categories. The principle of SLE severity classification takes into account the level of disease activity as well as its duration and in order to quantify SLE activity in the clinical situation, indices for SLE activity take into account both laboratory results and symptoms (Petri et al., 1992). The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) are presently using the improved classification criteria for diagnostic decisions for SLE (Ameer et al., 2022).

Despite the difficulties associated with the disease, research has shown that early detection of SLE reduces the risk of flare-ups, prompts use of healthcare services, and lessens the financial burden of the condition. Due to the self-replicating autoimmune phenomena caused by these factors, diagnosis becomes a significant issue that relies on clinical competence as phenomenon manifests itself in a variety of clinical presentations and changes with time. In clinical settings, serological measurements of autoantibodies - generally, the anti-nuclear autoantibodies (ANA) and specifically, the anti-double stranded (ds) DNA are routinely carried out of patients suspected to have SLE and this forms the diagnostic foundation for clinical intervention (Petri et al., 1992, Connelly and Morand, 2021). However, whether these serological measurements correlate with severity classification of SLE, especially among Iraqi patients, remains to be established. Here, we investigated the relationship between serological measurements of autoantibodies and complement proteins with severity classification of SLE patients.

2. Materials, Patients and Methods

2.1.Study Design and Subjects

This study was a cross-sectional study on patients clinically diagnosed with SLE by a specialist and were attending the Al - Imam Husain Medical City in Kerbala Governorate and Marjan Medical City in Babylon Governorate, Iraq, at the Rheumatology and Nephrology clinics in these hospitals. The patients were of both sexes with ages ranging from 16 to 65 years with duration of disease between 1 to 15 years. For the purpose of comparison, age-matched non-SLE individuals (control) were included in the study. These participants had no family history of SLE and were without any apparent medical disease. All subjects included in this study were Arab Iraqis and the study was carried out from November, 2022 to August, 2023.

Ethical approval for human studies was sought and obtained from the research and ethics committee of the College of Medicine, University of Kerbala in Iraq. All subjects (SLE patients and control group) were informed about the study and its aims, and their consent were obtained.

2.2.Classification of SLE Severity

All SLE patients fulfilled the American College of Rheumatology (ACR) criteria for classification of SLE, and were sub-divided into 3 groups based on their SLE disease activity index (SLEDAI) score (mild 0 - 5, moderate 6 - 12, and severe > 12) (Aringer et al., 2019).

2.3.Exclusion Criteria

SLE patients as well as non-SLE participants with autoimmune diseases, inflammation, pregnancy, malignant tumors, neurological disorders and a history of other connective tissue diseases (such as homocystinuria, Marfan syndrome, rheumatic fever, rheumatoid arthritis and osteoarthritis) were excluded from the study.

2.4.Data and Sample Collection

The study data was collected with strict adherence to standard health and safety measures. Demographic and clinical data (such as name, age, sex and medical history) were collected via an interview which was done to patients and /or their parents through a questionnaire.

Blood samples were obtained by venipuncture following disinfection of the antecubital fossa with 70% ethanol. Four milliliters blood was drawn into a gel tube for serum preparation respectively. Serum was prepared by centrifugation at 3000 rpm for 15 minutes and the supernatants were dispensed into Eppendorf tubes and stored at -20^{o}C until use.

2.5.Measurement of Autoantibodies and Complement Proteins

For ANA and anti-dsDNA, enzyme-linked immunoassay testing was performed on the EUROIMMUN analyzer I system (EUROIMMUN, Luebeck, Germany) using the Bio-Rad ANA ELISA (Bio-Rad; Hercules, CA, USA) and anti-dsDNA-NcX enzyme-linked immunoassay (Nunc, Roskilde, Denmark) respectively, as per manufacturer's guidelines.

Following the manufacturer's instructions, serum C3c and serum C4 were evaluated using fully automated turbidimetric immunoassay on a cobas® c502 analyzer (Roche Diagnostics). For C3 and C4, the assay's sensitivity was 0.04 and 0.02 g/l, respectively. For C3 and C4, the reference ranges were 0.9–1.8 g/l and 0.1–0.4 g/l, respectively

3. Statistical Analyses

The statistical software SPSS-25 (Statistical Packages for Social Sciences, version 25) was used for data analysis. Simple frequency, percentage, mean, and standard error of mean were used to present the data. The Pearson's Chi-square test ($\chi 2$-test) was used to determine the significance of the difference in different percentages (qualitative data), independent t test was used to statistical comparison of numerical data between the study groups while One Way ANOVA followed by Tukey's HSD was used to determine the significance of the numerical data differences between the different SLE disease severity classifications of SLE patients. Statistical significance was set at $p < 0.05$.

4. Results

4.1.Demographical and Clinical Characteristics of the Study Subjects

This study recruited a total of 120 individuals comprising of 60 patients clinically diagnosed with SLE and 60 non-SLE individuals (control) with matched demographic features as the SLE patients. Table 1 shows the demography and some clinical features of the study subjects. The SLE patients consisted of 36 females and 24 males while the non-SLE participants consisted of 28 females and 32 males. While the overall age of the subjects ranged from 16 to 67, majority of them (28.3%) were between 41 – 50 years age range, followed by 31 – 40 years age range (i.e. 22.5%), with only 7 subjects aged above 60 years. Comparison between the study groups with respect to age ranges was not significant ($p = 0.41$) as most of the subjects were between the ages of 31 to 50 years in both study groups.

The marital status of the study subjects shows that 43 (71.7%) SLE patients were married, while 17 (28.3%) patients were single and 52 (86.7%) non-SLE participants were married, with 8 (13.3%) single persons in the non-SLE group. The difference between the groups with respect to marital status was statistically significant with $p = 0.04$. Regarding family history of SLE, 44 (73.3%) SLE patients had history of SLE disease, while 16 (26.7%) of them do not. However, all 60 (100%) subjects of the non-SLE group (control) had no family history of SLE disease. The difference between the study groups with respect to family history was statistically significant with $p = 0.01$. Thirty-six (representing 60%) of the patients had mild SLE, while 19 patients (representing 31.7%) had moderate SLE and 5 patients (representing 8.3%) had severe SLE.

Table 1: Demography and Some Clinical Features of the Study Subjects

Features	All (n = 120)	SLE (n = 60)	Non-SLE (n = 60)	p-value
Age range, years (n (%)				
16 - 20	10 (8.3)	4 (6.7)	6 (10.0)	0.41
21 - 30	19 (15.8)	9 (15.0)	10 (16.7)	
31 - 40	27 (22.5)	15 (25.0)	12 (20.0)	
41 - 50	34 (28.3)	13 (21.7)	21 (35.0)	
51 - 60	23 (19.2)	14 (23.3)	9 (15.0)	
> 60	7 (5.8)	5 (8.3)	2 (3.3)	
Sex, n (%)				
Male	56 (46.7)	24 (40.0)	32 (53.3)	0.14
Female	64 (53.3)	36 (60.0)	28 (46.7)	
Marital Status, n (%)				
Single	25 (20.8)	17 (28.3)	8 (13.3)	0.04*
Married	95 (79.2)	43 (71.7)	52 (86.7)	
Family History of SLE, n (%)				
Yes	44 (36.7)	44 (73.3)	0 (0.0)	
No	76 (63.3)	16 (26.7)	60 (100.0)	
SLE Disease Severity, n (%)				
Mild	36 (60.0)	36 (60.0)	-	
Moderate	19 (31.7)	19 (31.7)	-	
Severe	5 (8.3)	5 (8.3)	-	
SLE; Systemic lupus erythematosus, *Statistically significant at $p < 0.05$				

4.2.Laboratory Analysis of Serum Autoantibodies and Complement Proteins

Table 2 summarizes the serum levels of ANA and anti-dsDNA as well as complements C3 and C4 of the SLE patients in comparison to the non-SLE subjects. For ANA, the SLE patients had the mean value of 8.79±2.04 IU/mL which was significantly higher compared to that of the control (non-SLE) which was 0.91±0.03 IU/mL ($p = 0.01$). For anti-dsDNA, the SLE patients had the mean value of 36.55±3.61 IU/mL which was also significantly higher compared to that of the control (non-SLE) i.e. 14.71±0.63 IU/mL ($p = 0.001$). This data is graphically presented in Fig.1.

Table 2: Mean Serum Levels of ANA and Anti-Dsdna As Well as Complements C3 And C4 Of the SLE Patients in Comparison to the Non-SLE Subjects

Variable	SLE (Mean ± SEM)	Non-SLE (Mean ± SEM)	p – value
Age, years	41.53±1.71	39.30±1.55	0.194
ANA, IU/mL	8.79±2.04	0.91±0.03	0.01*
Anti-dsDNA, IU/mL	36.55±3.61	14.71±0.63	0.001*
C3, g/L	1.09±0.19	2.86±0.09	0.04*
C4, g/L	0.3±0.02	1.10±0.04	0.02*
SLE; systemic lupus erythematosus, SEM; standard error of mean, ANA; anti-nuclear autoantibodies, Anti-dsDNA; anti-double stranded DNA, C; complement *Statistically significant at $p < 0.05$			

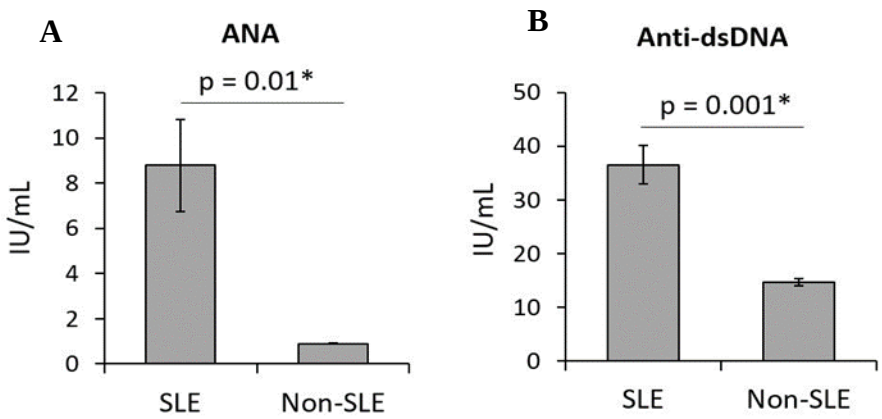

Figure 1: Mean Levels of Serum Autoantibodies. **(A)** The mean ANA level was significantly higher in the serum of SLE patients compared to the non-SLE group. **(B)** Similarly, the mean anti-dsDNA level was significantly higher in the serum of SLE patients compared to the non-SLE group ($p < 0.05$). *Statistically significant at $p < 0.05$. Error bars represent the standard error of the mean (SEM).

For C3, the SLE patients had the mean value of 1.09±0.19 g/L which was significantly lower compared to that of the control (non-SLE) which was 2.86±0.09 g/L ($p = 0.04$). For C4, the SLE patients had the mean value of 0.30±0.02 g/L which was significantly lower compared to that of the control (non-SLE) which was 1.10±0.04 g/L ($p = 0.02$). This data is graphically presented in Fig.2.

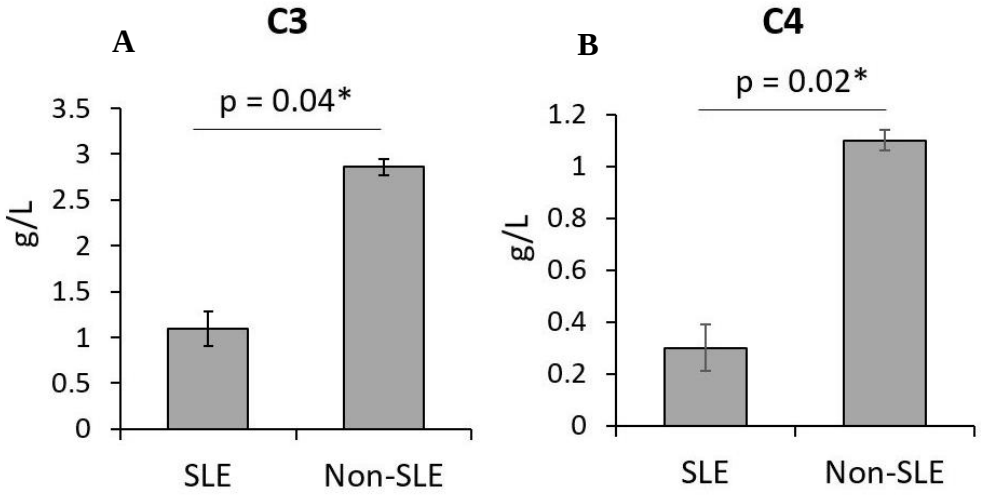

Figure 2: Mean Levels of Serum SLE-associated Complement. **(A)** Mean C3 level was significantly lower in the serum of SLE patients compared to the non-SLE group. **(B)** Similarly, the mean C4 level was significantly lower in the serum of SLE patients compared to the non-SLE group ($p < 0.05$). *Statistically significant at $p < 0.05$. Error bars represent the standard error of the mean (SEM).

4.3.Serum Autoantibodies and Complement Proteins Levels Based on SLE Disease Severity

Table 3 summarizes the serum levels of ANA and anti-dsDNA as well as complements C3 and C4 of the SLE patients with respect to their SLE disease severity classification. Levels of ANA increased with severity of disease as the patients with mild SLE had the mean value of 7.99±0.65 IU/mL, those with moderate SLE had 9.83±0.93 IU/mL and those with severe SLE had 13.70±1.60 IU/mL, the differences of which were statistically significant ($p = 0.004$). For anti-dsDNA, serum levels also increased with disease severity as the patients with mild SLE had the mean value of 33.38±2.18 IU/mL, those with moderate SLE had 39.32±2.28 IU/mL and those with severe SLE had 42.84±4.80 IU/mL, however, the observed differences were not statistically significant ($p = 0.101$). This data is graphically presented in Fig.3.

Table 3: The SLE Patients' Mean Serum Levels Of ANA, Anti-Dsdna, and Complements C3 And C4 in Relation to their SLE Severity Classification

Variable	SLE Disease Severity			p – value
	Mild (Mean ± SEM)	Moderate (Mean ± SEM)	Severe (Mean ± SEM)	
Age, years	42.50±1.36	41.21±1.99	46.40±2.77	0.455
ANA, IU/mL	7.66±0.65	9.83±0.93	13.70±1.60	0.004*
Anti-dsDNA, IU/mL	33.38±2.18	39.32±2.28	42.84±4.80	0.1
C3, g/L	1.19±0.05	0.98±0.11	0.72±0.17	0.009*
C4, g/L	0.32±0.02	0.29±0.03	0.16±0.03	0.002*
SLE; systemic lupus erythematosus, SEM; standard error of mean, ANA; anti-nuclear autoantibodies, Anti-dsDNA; anti-double stranded DNA, C; complement, *Statistically significant at $p < 0.05$.				

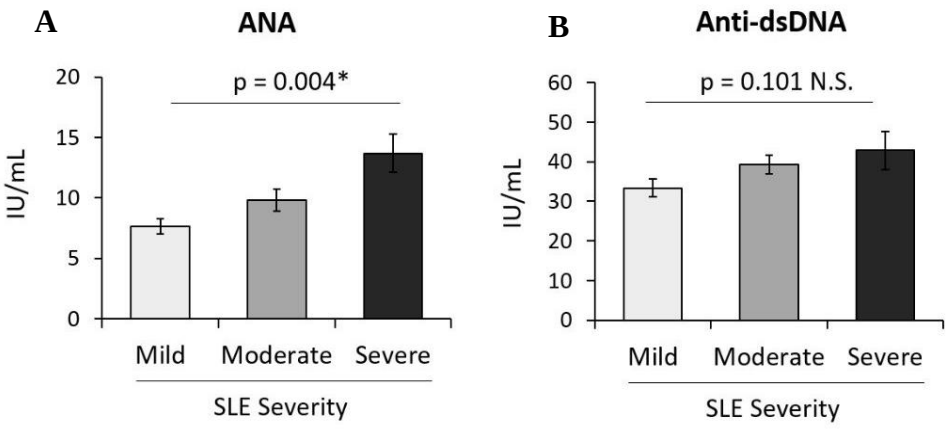

Figure 3: Mean Serum Autoantibodies of SLE Patients Based on Disease Severity Classification. **(A)** Mean ANA levels based on disease severity classification. **(B)** Mean anti-dsDNA levels based on disease severity classification.

In SLE patients, levels of ANA increased as the disease progressed; these differences were statistically significant ($p = 0.004$). For anti-dsDNA, serum levels also increased with disease severity as the SLE patients however, the observed differences were not statistically significant ($p = 0.101$). *Statistically significant at $p < 0.05$. Error bars indicated standard error of mean (SEM). Conversely, levels of C3 and C4 decreased with severity of disease as the patients with mild SLE had the mean C3 value of 1.19±0.05 g/L, those with moderate SLE had 0.98±0.11 g/L and those with severe SLE had 0.72±0.17 g/L, the differences of which were statistically significant ($p = 0.009$). For C4, serum levels also decreased with disease severity as the patients with mild SLE had the mean C4 value of 0.32±0.02 g/L, those with moderate SLE had 0.29±0.03 g/L and those with severe SLE had 0.16±0.03 g/L; the observed differences were statistically significant ($p = 0.002$). This data is graphically presented in Fig.4.

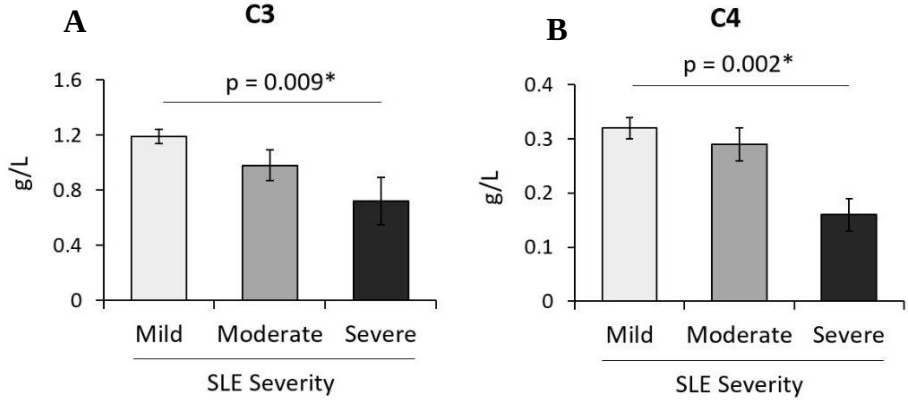

Figure 4: Mean Serum Complement Levels of SLE Patients Based on Disease Severity Classification. **(A)** Mean serum C3 levels declined with increasing disease severity (mild > moderate > severe), with a statistically significant difference ($p = 0.009$). **(B)** Mean serum C4 levels also declined with increasing disease severity, with a statistically significant difference ($p = 0.002$). *Statistically significant at $p < 0.05$. Error bars represent the standard error of the mean (SEM).

5. Discussion

The complexity and heterogeneity of SLE make it challenging to assess the disease's severity using a single serological indicator. One of the hallmarks of SLE is increased apoptosis along with impaired clearance of apoptotic cells, resulting in the emergence of high levels of a myriad of autoantibodies. Autoantibody synthesis also leads to tissue harm through the formation and deposition of autoantibody-autoantigen immune complexes (Ding et al., 2023). The EULAR/ACR SLE classification criteria for SLE have been developed and updated regularly to aid diagnosis, monitoring and management of SLE (Aringer et al., 2019). However, factors such as race, locality, and sex affect the severity, risk, and clinical manifestation of SLE, with women and some non-European-derived populations having a higher prevalence (Bertsias et al., 2010). To relate the severity classification of SLE with levels of some serological indicators, this study measured the serum levels of SLE-associated autoantibodies (ANA and anti-dsDNA), complement components (C3 and C4), of SLE patients in comparison to non-SLE subjects (control). The serum levels of the patients' autoantibodies complement components were the evaluated based on their disease severity classification.

To achieve homogeneity in the study population, we ensured that the SLE patients as well as the non-SLE participants recruited in the study were Iraqi Arabs. Also, statistical comparisons between the study groups with respect to mean age and age range, were not significant ($p < 0.05$). However, as reported in previous studies (Margery-Muir et al., 2017, Nusbaum et al., 2020), female preponderance (60%) was observed in the present study. Interestingly, marriage status of the SLE patients and the non-SLE group differed significantly, according to the results ($p < 0.05$). A crucial observation to make is that, while the difference between the groups in married versus single was marginally significant ($p = 0.04$), this finding contradicts the findings of Qu et al.'s study, which found that these differences were not statistically significant (i.e. $p = 0.058$, p-value set at > 0.05) (Qu et al., 2019). On the other hand, our finding suggests that the marital status bears some responsibility for SLE, and may have an assessment role in the condition in future studies. The role of family history of SLE in the predisposition to development of SLE has been extensively evaluated in previous studies. In 2002, Cooper et al identified family history of lupus or other systemic autoimmune diseases in a parent or sibling as a risk factor for development of lupus (Cooper et al., 2002). Similar results have been reported in more recently conducted studies. Here, we observed that 73% of the SLE patients had family history of the disease while none of the non-SLE subjects had family history of the disease. Moreover, a meta-analysis reported deleterious impact of familial history on the clinical manifestations and laboratory disorders in SLE patients (Chen et al., 2018).

Categorization of the SLE patients based on SLEDAI severity scores, indicated that majority of the patients (60%) had mild SLE, with only 5 patients having severe levels of the disease. This finding is consistent with the reports of many SLE severity classification studies (Fanouriakis et al., 2020, Nikolopoulos et al., 2020).

The majority of lupus patients have a mild form of the disease that is typified by flares, which are periods of time when symptoms worsen for a while, then get better or perhaps go away entirely. However, it has been highlighted that

some cases may become more severe over time, with mild, moderate, and severe cases eventually making up one-third of each group (Fanouriakis et al., 2020).

SLE patients are characterized by the present of autoantibodies, which can form immune complexes and for this reason are considered harmful. The development of antibodies to components of the cell nucleus, also known as anti-nuclear antibodies, or ANA, is a common serological finding among these SLE manifestation. With antibodies to DNA and Sm, a compound of proteins and uridine-rich RNA molecules that is strongly linked to SLE, these antibodies target DNA, RNA, proteins, and protein-nucleic acid complexes (Pisetsky et al., 2019).

In this study, patients with SLE had mean serum ANA and anti-dsDNA levels significantly higher than those without SLE. This is consistent with the findings of previous studies (Elessawi et al., 2019, González et al., 2015).

Positive ANA was initially not a prerequisite for admission in earlier SLE classifications; it was seen as equally significant as anti-dsDNA and other autoantibodies. With anti-dsDNA autoantibodies listed as one of the classification criteria for SLE, they are relatively effective indicators for monitoring disease activity and treatment response (Aringer et al., 2021). Additionally, this study demonstrates a significantly lower level of C3 and C4 cytokine markers in the SLE patients when compared to Control ($p = 0.02$ and 0.04, respectively). These results are in conflict with those of Troldborg et al, who found that SLE patients had higher plasma concentrations of C3 than controls ($p < 0.05$) (Troldborg et al., 2018). However, they are in line with Qu et al. (2019), who found that SLE was associated with lower levels of C4 and C3 (0.19±0.08 g/L and 0.58±0.24 g/L, respectively), when compared to a healthy control (0.29±0.11 g/L and 0.97±0.15 g/L) ($p < 0.001$). In general, Sandhu and Quan noted that complement activation is important in SLE and that blood levels of C3 and C4 should be continuously monitored to determine whether the disease is active (Sandhu and Quan, 2017).

Serum levels of the autoantibodies and complement components may play significant role in the diagnosis of SLE, however due to the heterogenous nature of the disease, their predictive usefulness in monitoring disease severity and progression remains to be established. In our findings, elevations in ANA levels along with decrease in C3 and C4 levels were observed to occur significantly in relation to SLE disease severity as it worsens from mild to moderate and to severe. However, while there were observable elevations in anti-dsDNA with increase in disease severity, these differences were not statistically significant. This observation highlights the significance of ANA evaluation in the diagnosis and management of SLE and supports the inclusion of a positive result in the ANA detection test as a required entry condition for the SLE classification, which is one of the primary modifications made to the EULAR/ACR 2019 classification criteria in comparison to earlier SLE classification systems (Aringer et al., 2021). Some authors have even referred to this as a strategic change, calling it the most relevant change made by the EULAR/ACR 2019 (Damoiseaux and van Beers, 2023, Serra-García et al., 2022). Anti-dsDNA on the other hand was initially regarded the most prominent immunological criterion in the EULAR/ACR 2019 classification once the positive ANA entry criterion is satisfied. However, recent studies have raised questions on the specificity of anti-dsDNA as a pathogenic factor and biomarker for SLE. This resulted from reports of anti-dsDNA antibodies among patients with bacterial, viral or parasitic infections (Wozencraft and Staines, 1990, Hamilton et al., 2006, Rekvig et

al., 2006) as well as cancers (Lv et al., 2005) and even healthy individuals (Rekvig, 2015). The result we obtained here supports the growing realization that the anti-dsDNA is not a distinct SLE-specific molecule and hence the usefulness of anti-dsDNA determination in the diagnosis and classification of SLE should be investigated further and followed up.

There is contradiction in the relationship between SLE and the complement system. It has long been established that SLE exacerbations stimulate the complement system, which is thought to be mostly due to nephritic activity. Whether this complement activation has a role in the pathophysiology of SLE or is just a harmless event has been discussed (Weinstein et al., 2021). As a modulator of inflammation, complement insufficiency increases the risk of developing SLE. While C3 deficit is infrequently linked to SLE-like illness, inherited complement C4 deficiency, whether partial or total, carries a substantial risk of developing SLE (Walport et al., 1997). Nevertheless, these complement components play a crucial role in avoiding immune complex-mediated tissue damage, as evidenced by the correlation found between complement deficits and SLE (Narayanan et al., 2010). Importantly, this study underscores the potential significance of complement components C3 and C4 determination not only in the diagnosis of SLE, but also in management of the disease.

6. Conclusion

In conclusion, we have demonstrated that although ANA and anti-dsDNA autoantibodies may be important in the diagnosis of SLE, their use in predicting the severity of the disease varies considerably, with ANA having a higher specificity than anti-dsDNA. Additionally, we emphasized the significance of complement components C3 and C4 in monitoring and forecasting the severity of the disease in addition to their role in the diagnosis of SLE.

Since the SLE autoimmune response is heterogenous, it may never be possible to develop a diagnostic and severity evaluation approach that is 100% sensitive and specific. Consequently, it is important to define reasonable goals that can be applied universally in all clinical settings. Further research is required to enhance the diagnostic and disease management efficacy of anti-dsDNA assays in diverse populations.

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