DOI: 10.24996/ijs.2024.65.10.17

Novel Insights Into The Role Of Serum Interleukin-39 In Patients With Systemic Lupus Erythematosus

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Received: 28/5/2023 Accepted: 24/8/2023 Published: 30/10/2024

Abstract
Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that has a genetic predisposition and a complex pathogenesis in which the involvement of various cytokines has been shown. In the current study, serum interleukin-39 (IL-39) levels from 99 patients with SLE and 33 healthy control subjects who attended the Baghdad Teaching Hospital Rheumatology Unit were examined. Patients were divided into 3 subgroups according to disease status: inactive (n = 33), active moderate (n = 33), and active severe (n = 33). This topic has not yet been explored, so the significance of IL-39 as a biomarker for SLE was evaluated. Cytokine levels were measured using enzyme-linked immunosorbent assay kits. Full medical histories, body mass index, gender, and clinical disease activity, the latter evaluated using the SLE disease activity index, were documented. Laboratory disease parameters, including anti-dsDNA antibodies, C3 and C4 levels, erythrocyte sedimentation rate, and C-reactive protein titres, were measured. The mean age of patients was 33.92 ± 0.91 years. The IL-39 level was higher in patients (13.70 ± 0.35 ng/l) than in controls (10.67 ± 0.19 ng/l, $p < 0.01$). The mean of IL-39 levels was highest in patients with active severe SLE (17.42 ± 0.48 ng/l) and then became incrementally lower with reducing disease severity, i.e., active moderate, 13.34 ± 0.23 ng/l; inactive, 10.93 ± 0.24 ng/l. Serum IL-39 showed good validity for the diagnosis of SLE. With a cut-off value ≥ 10.25 ng/l and an area under the curve of 0.79, diagnostic sensitivity, accuracy, and specificity were 79.98%, 51.5%, and 71.97%, respectively. In conclusion, serum IL-39 levels were significantly higher in patients with SLE than in healthy controls and were correlated with disease activity. This interleukin may be useful in predicting disease severity.

Keywords: IL-39, systemic lupus erythematosus, SLE, autoimmune disease.

رؤى جديدة حول دور IL-39 في مصل مرضى الذئبة الاحمراري الجهازي

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الخلاصة
داء الذئبة الاحمراري الجهازي (SLE) هو مرض مناعي ذاتي مزمن له استعداد وراثي وإمراضية معقدة حيث تظهر الحركيات الخلوية المختلفة دورا مهما في تكوينه. في الدراسة الحالية، تم فحص مستويات (انترلوكين39) في المصل من 99 مريضا يعانون من مرض داء الذئبة الاحمراري الجهازي و33 شخصا من

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الأصحاء الذين حضروا الى وحدة أمراض الروماتيزم في مستشفى بغداد التعليمي. تم تقسيم المرضى إلى 3 مجاميع فرعية وفقًا لحالة المرض، غير نشط (عددهم 33)، نشط معتدل (عددهم 33) ونشط شديد (عددهم 33). لم يتم استكشاف هذا الموضوع حتى الان ولذلك تم تقييم أهمية IL-39كمؤشر حيوي لمرض داء الذئبة الاحمراري الجهازي، تم قياس مستويات الحركيات الخلوية باستعمال تقنية الامتصاص المناعي المرتبط بالأنزيم. تم توثيق التاريخ الطبي الكامل ومؤشر كتلة الجسم والجنس ونشاط المرض السريري، وتم تقييم الاخير باستعمال مؤشر نشاط مرض داء الذئبة الاحمراري الجهازي. تم قياس عدد من معايير المرض المختبرية مثل الأجسام المضادة لـ dsDNA ومستويات C3و C4ومعدل ترسيب كريات الدم الحمراء ومستوى البروتين التفاعلي C.وكان متوسط اعمار المرضى (33.92 ±0.91) سنة. ظهر مستوى IL-39 اعلى في المرضى من مستواه في المجموعة الصحية الضابطة 13.70 ±0.35 نانوغرام /لتر مقابل 10.67 ± 0.19 نانوغرام / لتر، بمستوى الاحتمالية <0.01). وكان متوسط مستوىIL-39 عالي في مصول المرضى الذين يعانون من مرض داء الذئبة الاحمراري الجهازي الشديدة النشطة (17.42 ± 0.48 نانوغرام /لتر)، ثم انخفض بشكل تدريجي مع انخفاض شدة المرض في الحالات المتوسطة النشطة 13.3411 ± 0.23153 نانوغرام / لتر؛ غير النشطة، 10.93 ± 0.242 نانوغرام / لتر. أظهرمستوى مصل IL-39 صلاحية جيدة لتشخيص مرض داء الذئبة الاحمراري الجهازي. مع قيمة قطع ≤ 10.25 نانوغرام / لتر، ومنطقة تحت المنحنى 0.79، كانت الحساسية التشخيصية والدقة والنوعية 79.98٪ و51.5٪ و71.97٪ على التوالي .
ختاما، كانت مستويات IL-39 في المصل أعلى في مرضى داء الذئبة الاحمراري الجهازي مقارنة بالمجموعة الضابطة الصحية وبشكل ملحوظ، ومرتبطة بنشاط المرض. IL-39 ربما يكون ذو قيمة في توقع شدة المرض.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, systemic autoimmune disease characterized by inflammation and abnormal immune system activity, which lead to an insufficient immune tolerance of autoantigens [1, 2]. A complex disorder, SLE is associated with diverse immunological pathways that result in a wide variety of clinical manifestations, such as renal, cardiovascular, or dermatological complications [2]. Such heterogeneity presents significant challenges for the diagnosis, classification, and treatment of the disease [3, 4]. The diagnosis and monitoring of SLE disease activity are therefore dependent on the analysis of clinical symptoms and the use of supportive serological and biochemical investigations [4]. It may, however, be challenging to distinguish between SLE and other disorders with similar symptoms [5]. Reliable biomarkers that accurately reflect disease activity and enable this differentiation are vital, particularly as early diagnosis improves patient prognosis. These biomarkers must also be able to reflect the degree of immune activity and inflammation within the body [2, 5]. Despite medical advancements in the management and treatment of SLE, the ever-increasing burden, associated risk of mortality, and current limited understanding of SLE highlight an urgent need for research into its pathogenesis [2, 4, 6]. Additionally, the discovery of non-invasive biomarkers is critical to improving the early diagnosis of SLE and the development of effective therapeutic agents [1, 2].

Immunological markers have been under investigation in order to assist with the early detection of SLE and to further understand its pathological processes [4]. Several studies have proposed the use of cytokines as biomarkers for disease activity. Cytokines are soluble, low-molecular-weight glycoproteins involved in regulating various aspects of the immune response and, importantly, inflammatory reactions. Cytokines have also been heavily implicated in the pathogenesis of SLE and may be a promising tool for monitoring and diagnosing the disease [4].

Interleukins are a type of cytokine frequently used as biomarkers of disease progression and for monitoring various conditions. Interleukin (IL)-6 and IL-8 have been demonstrated to be useful biomarkers for severe COVID-19 and disease prognosis [7–9]. More relevantly, the levels of several interleukins have been associated with various forms of SLE, such as neuropsychiatric SLE. Many studies have correlated IL-6 and IL-10 levels with SLE disease activity and demonstrated that these interleukins are of value for the assessment of serological and clinical disease status [4, 10]. More recently, the IL-12 family, which has four members, has been under investigation owing to their clinical relevance in multiple disorders and their involvement in pro-inflammatory responses [11, 12].

IL-39 is a newly discovered cytokine from the IL-12 family. This heterodimer glycoprotein is made up of two covalently linked subunits, i.e., α and β chains [13, 14]. The IL-12 family is involved in the regulation of immune system function, differentiation, and inflammatory response mediators [11, 12, 14]. Although the number of studies that have investigated IL-39 is limited, its potential as a biomarker in a range of pathologies has been demonstrated, and this cytokine has also been implicated in the pathogenesis of inflammatory autoimmune diseases [15]. Increased IL-39 levels and IL-39 upregulation have been reported in studies on ankylosing spondylitis, which could enable differentiation between patients with and without the condition [15]. IL-39 has also been observed to be upregulated in patients with neuromyelitis optica spectrum disorders, acute coronary syndrome, type 2 diabetes mellitus, and chronic graft-versus-host disease, results that have highlighted its potential to act as an indicator of immunological and systolic dysfunction [13, 15, 16].

IL-39 has been identified as a mediator of pro-inflammatory responses in mouse models. It exhibited pro-inflammatory effects in lupus-like mice through the activation of STAT1 and STAT3 signaling pathways [13, 15]. In addition, these studies revealed the secretion of IL-39 by B-cells and high IL-39 expression in lupus-like mice following stimulation with lipopolysaccharides [14]. Additional immune cells were also found to express IL-39 mRNA, and the activation of $GL7^{+}B$ and $CD138^{+}$ plasma cells promoted IL-39 expression. Given the association of this cytokine with inflammatory pathways, particularly as demonstrated by these models in SLE, and since inflammation is a hallmark of SLE, there may be significant value in investigating the diagnostic potential of IL-39 in more detail [13]. These studies additionally suggest that IL-39 makes a possible contribution to the immunopathogenic mechanisms that occur in SLE [14].

Despite these promising results, the involvement of IL-39 in human systems is still contested due to a lack of sufficient evidence. Some studies consider IL-39 to be just a theoretical concept, whereas others suggest that IL-39 production only occurs in mice [13]. However, in a small number of studies, detectable levels of this cytokine have been demonstrated in humans, suggesting that the relevance of IL-39 merits further investigation [13].

The use of other cytokines as biomarkers for inflammatory autoimmune diseases and the demonstrated link between these cytokines, the pathogenesis of SLE, and the associated inflammatory pathways highlight the potential diagnostic value of IL-39. Studies that endeavor to determine the relationships between IL-39 and other cytokines, as well as their biological function, effects, and mechanisms in humans, particularly for inflammatory diseases such as SLE, can therefore provide vital information [14]. An improved understanding of the role of IL-39 and its function may facilitate the further classification of SLE as well as allow its pathological complexity to be further delineated. Ultimately, useful biomarkers that could be utilized for the early diagnosis of SLE may be identified.

2. Materials and Methods

2.1 Patients and controls

From November 2022 to January 2023, blood samples were collected from 99 patients with SLE and 33 control subjects who attended the Rheumatology Department in Baghdad Teaching Hospital. Patients were divided into three subgroups according to SLE disease status: inactive (n = 33); active moderate (n = 33); and active severe (n = 33). The SLE patients comprised 97 females and 2 males, with an age range of 18–60 years. The control group included 31 females and 2 males and had an age range of 20–60 years. Patients older than 18 years with an active disease state who had been diagnosed in accordance with the 2019 European League Against Rheumatism/American College of Rheumatology classification guidelines for SLE met the inclusion criteria [17]. Individuals with comorbidities, additional connective tissue conditions, seronegative spondylarthritis, malignancy, pregnancy, or who refused to participate were excluded.

Under the supervision of a rheumatologist, an information sheet and consent form were completed for each patient. The data collected encompassed age, gender, body mass index (BMI), clinical disease activity as evaluated using the SLE disease activity index, and type of current therapy. Anti-dsDNA antibodies, C3 and C4 levels, C-reactive protein (CRP) titres, and erythrocyte sedimentation rate (ESR) were some of the disease parameters tested in the lab. Information regarding the study objectives and procedures was provided to all participants, and formal consent was sought prior to commencing the study. The Scientific Ethics Committee of the University of Baghdad College of Medicine approved the study. The immunological tests were performed at the International Center for Research and Development.

Patients with SLE receive therapy according to the severity of their illness. Individuals with mild disease, e.g., involvement of the skin, joints, and mucosa, are treated with hydroxychloroquine, 200–400 mg/day. Although some individuals may not require any extra therapy, glucocorticoids, i.e., 7.5 mg/day, may also be needed. Moderate illness is non-life-threatening and comprises constitutional, cutaneous, musculoskeletal, or hematological manifestations. These often respond to hydroxychloroquine, 200–400 mg/day, or chloroquine plus short-term prednisone or similar agents. After hydroxychloroquine takes effect, the prednisone dose is decreased. Azathioprine, 2–3 mg/kg/day, and rituximab, 500–1000 mg/2 weeks, are then given.

2.2 Immune assays

Blood samples were collected in gel tubes and then stored at -20 °C. The serum was extracted by centrifuging the samples at 1000–3000 rpm for 10 minutes. In order to find anti-IL-39 antibodies in human serum, the manufacturer's instructions (Sun Long Biotech Company, China) were followed, and an enzyme-linked immunosorbent assay (ELISA) was used. A plate reader was used to measure the absorbance at a wavelength of 450 nm.

2.3 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences, version 21.0 (SPSS, IBM). Independent sample t-tests, analysis of variance (ANOVA), and less significant difference (LSD) tests were performed for intergroup comparisons of the quantitative variables, i.e., age, BMI, and IL-39. Normally distributed data were expressed as mean ± standard deviation (SD). A Pearson chi-square test ($\chi 2$) was applied for comparisons of qualitative variables, i.e., age and BMI. A binomial Z-test was performed for comparison of gender and treatment intake. A Pearson correlation test was used to identify any relationships between serum IL-39 levels and age, BMI, duration of SLE disease, ESR, C3, and C4 levels. The validity of the ELISA test was estimated via a receiver operator characteristic curve. Using a cut-off value, diagnostic performance parameters assessed included the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. The statistical significance threshold accepted was P < 0.05.

3. Results and Discussion

As a member of the cytokine IL-12 family, IL-39 has the potential to be a critical regulator of immune and inflammatory responses [15, 18]. Studies conducted on lupus-like mice have provided vital information about the potential function of IL-39 [4, 14, 19]. As the role of IL-39 in humans remains contested, further studies are required in order to clarify its role [18]. The current research has therefore sought to evaluate the use of IL-39 as a diagnostic biomarker of SLE in humans by conducting serum analysis on blood samples from SLE patients and healthy individuals. Also, the parameters that were looked at were compared with IL-39 levels to see how well IL-39 works as a biomarker for diagnosing SLE and keeping an eye on the disease. The control group was age- and sex-matched to the SLE patient cohort in order to enable an adequate intergroup comparison to be carried out.

3.1. Characteristics of patients

A demographic comparison of SLE patients' groups and controls was performed. As shown in Table 1, no significant differences were seen between patients and control groups in terms of gender, i.e., SLE patients: female, 92 (92.93%), male, 7 (7.07%); controls: female, 31 (93.9%), male, 2 (6.1%).

The highest numbers of patients with SLE were seen in the age groups 31–40 years (42, 42.42%) and 19–30 years (35, 35.36%). These statistics were reflected in the age-matched controls, i.e., 31–40 years, 10 (30.3%), and 19–30 years, 12 (36.43%). The mean ages of the two studied groups were similar, i.e., controls, 34.42 ± 1.82 years; SLE patients, 33.92 ± 0.91 years.

Most participants were in the overweight category, i.e., controls: 19 (57.6%); SLE patients: 40 (40.4%). The remainder were found to be obese, i.e., controls, 7 (21.2%); SLE patients, 33 (33.3%), or of normal weight, i.e., controls, 7 (21.2%); SLE patients, 26 (26.3%). No differences in BMI distribution were seen between the two groups.

The SLE patients showed a trend towards a higher mean BMI than the controls, i.e., 28.31 ± 0.571 kg/m^2 vs. 25.68 ± 0.74 kg/m^2, but this failed to reach significance.

Table 1: Demographics and other parameters: distributions within the two studied groups, i.e., SLE patients and controls

Parameters		Studied groups: Controls N = 33	Studied groups: Patients N = 99	***P* - value**
Gender	**Male**	**2 (6.1%)**	**7 (7.07%)**	**0.24**
	Female	**31 (93.9%)**	**92 (92.93%)**	
Age-groups (years)	**19 - 30**	**12 (36.4%)**	**35 (35.36%)**	**0.22**
	31 - 40	**10 (30.3%)**	**42 (42.42%)**	
	41 - 50	**8 (24.2%)**	**20 (20.2%)**	
	51 - 60	**3 (9.1%)**	**2 (2.02%)**	
BMI groups	**Normal weight**	**7 (21.2%)**	**26 (26.3%)**	**0.21**
	Overweight	**19 (57.6%)**	**40 (40.4%)**	
	Obese	**7 (21.2%)**	**33 (33.3%)**	
Age (years)	**Mean**	**34.42**	**33.92**	**0.79**
	Std. Error	**1.82**	**0.91**	
BMI (kg/m^2)	**Mean**	**25.68**	**28.31**	**0.09**
	Std. Error	**0.74**	**0.57**	

Following testing with ANOVA, no significant differences were found for either mean BMI or disease duration between the different patient groups (Table 2).

The mean BMI was equivalent in all 3 patient groups: active severe, 28.65 ± 1.08 kg/m^2; active moderate, 28.32 ± 1.12 kg/m^2; and inactive, 27.92 ± 0.86 kg/m^2. The LSD test indicated no differences amongst intergroup comparisons, i.e., inactive vs. active moderate, $P = 0.71$; inactive vs. active severe, $P = 0.55$; active moderate vs. active severe, $P = 0.81$.
No differences in disease duration were observed between patient groups: active moderate, 6.75 ± 1.18 years; active severe, 5.68 ± 0.76 years; and inactive, 4.61 ± 0.83 years ($P = 0.19$). The LSD test showed no intergroup comparison significance, i.e., inactive vs. active moderate, $P = 0.25$; inactive vs. active severe, $P = 0.47$; active moderate vs. active severe, $P = 0.64$.

However, highly significant differences were observed with respect to additional parameters, although there were some exceptions.
The mean of ESR test values was maximal in the blood of patients; it was 47.58 ± 5.73 in patients with active severe disease and became incrementally lower in individuals with active moderate (41.13 ± 4.85) and inactive (23.64 ± 2.48) disease ($P = 0.002$). A similar P-value was obtained following a LSD test, with the exception of the comparison between active moderate vs. active severe disease states ($P = 0.42$).

The mean anti-dsDNA antibody levels were markedly increased in patients with active severe SLE (91.16 ± 23.62) when compared to those with active moderate (32.95 ± 3.43) and inactive (20.323 ± 0.8457) disease states ($P = 0.0007$). Similar results were obtained for the LSD test, with the exception of the comparison between inactive and active moderate groups ($P = 0.65$).

The mean C3 levels were reduced in patients with active severe SLE (0.66 ± 0.08), a value that was less than that seen in active moderate disease (0.75 ± 0.07), which again was lower than in the cohort with inactive disease (1.15 ± 0.09, $P = 0.0009$). This significance level was present for all intergroup comparisons except for those between patients with active moderate and active severe disease states ($P = 0.33$).
The mean of C4 levels increased incrementally with disease severity, i.e., active severe, 0.06 ± 0.01; active moderate, 0.27 ± 0.037; and inactive, 0.29 ± 0.015. The LSD tests were highly significant ($P < 0.01$), apart from the comparison between inactive vs. active moderate disease states ($P = 0.51$).

Table 2: Mean distributions of patient parameters within SLE patient groups

SLE patient groups		Mean	Std. Error	LSD test (*P* – Value)	
BMI (kg/m^2)	**Inactive**	**27.93**	**0.86**	**A**	**0.71**
	Active moderate	**28.32**	**1.12**	**B**	**0.55**
	Active severe	**28.65**	**1.08**	**C**	**0.81**
	ANOVA test (P – Value): *P* = 0.27				
Duration (years)	**Inactive**	**4.61**	**0.83**	**A**	**0.25**
	Active moderate	**6.75**	**1.18**	**B**	**0.47**
	Active severe	**5.68**	**0.76**	**C**	**0.64**
	ANOVA test (*P* – Value): *P* = 0.19				
ESR	**Inactive**	**23.64**	**2.48**	**A**	**0.00004**
	Active moderate	**41.13**	**4.85**	**B**	**0.0007**
	Active severe	**47.58**	**5.71**	**C**	**0.42**
	ANOVA test (*P* – Value): *P* = 0.002				
Anti-dsDNA antibodies	**Inactive**	**20.32**	**0.85**	**A**	**0.65**
	Active moderate	**32.95**	**3.43**	**B**	**0.01**
	Active severe	**91.16**	**23.62**	**C**	**0.001**
	ANOVA test (*P* – Value): *P* = 0.0007				
C3	**Inactive**	**1.15**	**0.09**	**A**	**0.0009**
	Active moderate	**0.75**	**0.07**	**B**	**0.004**
	Active severe	**0.66**	**0.08**	**C**	**0.31**
	ANOVA test (*P* – Value): *P* = 0.003				
C4	**Inactive**	**0.29**	**0.02**	**A**	**0.51**
	Active moderate	**0.27**	**0.04**	**B**	**0.0008**
	Active severe	**0.06**	**0.01**	**C**	**0.0001**
	ANOVA test (*P* – Value): *P* = 0.001				

A = inactive vs. active moderate; B = inactive vs. active severe;
C = active moderate vs. active severe.

The data in Table 3 show that the distributions of CRP and treatment intake are related to the severity of SLE, i.e., DMARD intake (P = 0.14). inactive: yes, 30 (90.9%), no, 3 (9.1%); active moderate: yes, 28 (84.8%), no, 5 (15.2%); active severe: yes, 24 (72.7%), no, 9 (27.3%).

Significant differences (*P* = 0.04) were noted for the CRP results, i.e., inactive: positive, 2 (6.06%), negative, 31 (93.94%); active moderate: positive, 3 (9.1%), negative, 30 (90.9%); active severe: positive, 7 (21.21%), negative, 26 (78.79%).

Highly significant differences for alternative treatment intake types were also observed, i.e., steroid intake: inactive: yes, 11 (33.3%), no, 22 (66.7%); active moderate: yes, 24 (72.7%), no, 9 (27.3%); active severe: yes, 28 (84.85%), no, 5 (15.15%) (*P* = 0.0008). Similar results were obtained for biologic intake (Etanercept or Adalimumab): Active moderate: yes, 2 (6.06%), no, 31 (93.94%); active severe: yes, 11 (33.3%), no, 22 (66.7%) (*P* = 0.01).

Table 3: C-reactive protein (CRP) and treatment intake distributions within SLE patient groups

Parameters		Severity of SLE			*P* - Value
		Inactive N = 33	Active moderate N = 33	Active severe N = 33	
CRP	Positive	2 (6.06%)	3 (9.1%)	7 (21.21%)	0.04
	Negative	31 (93.94%)	30 (90.9%)	26 (78.79%)	
DMARDs intake	Yes	30 (90.9%)	28 (84.8%)	24 (72.7%)	0.14
	No	3 (9.1%)	5 (15.2%)	9 (27.3%)	
Steroid intake	Yes	11 (33.3%)	24 (72.7%)	28 (84.85%)	0.0008
	No	22 (66.7%)	9 (27.3%)	5 (15.15%)	
Biologics intake	Yes	-	2 (6.1%)	11 (33.3%)	0.01
	No	-	31 (93.9%)	22 (66.7%)	

The blood serum analysis included diagnostic parameters typically used for SLE, i.e., ESR, anti-dsDNA antibodies, C3, C4, and CRP levels. Both ESR and anti-dsDNA antibody levels went up with the severity of the disease. The highest levels of both were seen in the active-severe SLE group. The ESR is a measure of the sedimentation rate of red blood cells (RBC) in a tube. Higher blood protein levels are often observed in inflammatory conditions, which cause RBC to stick together, which, in turn, results in faster sedimentation. Thus, higher ESR figures are often indicators of inflammatory conditions and active disease processes, as observed by these results [20].
Anti-dsDNA antibody serum levels fluctuate with disease activity. High antibody titres are a disease marker for SLE, as suggested by the results obtained in this study [21–24]. Both C3 and C4 levels decreased with increasing disease severity. These complement proteins can be used to gauge SLE disease activity, as reduced levels are often observed in SLE [25]. A higher percentage of patients with active severe SLE tested had a positive CRP test compared to those with inactive or active moderate SLE. CRP levels rise with the onset of inflammatory stimuli; persistently elevated CRP titres are often observed in SLE [26]. Thus, the results obtained from these assays align with the SLE diagnostic guidelines and facilitate a comparison with IL-39 levels in order to validate the use of the latter as a diagnostic biomarker.

The discrepancies identified between men and women within this cohort were significant; further cytokine studies may provide vital information regarding these differences [27, 28]. For instance, Beenakker et al. [29] examined the cytokine differences between men and women, and while their study was not selective for patients with SLE, it provided a basis for conducting gender-specific cytokine studies that may be insightful for SLE. The majority of SLE participants were women between the ages of 19 and 45 years, which is consistent with previous studies [30, 31].

The relationship between BMI and SLE has not been fully elucidated in the literature, although associations have been reported between a heightened risk of SLE and obesity, severe clinical manifestations and higher BMIs, and increased BMIs and SLE [32–36]. However, no link was found in the reviewed studies between BMI and SLE disease activity or disease incidence [37, 38]. The correlations between BMI and IL-39 levels, or SLE, cannot be ascertained from the current data, and so further studies are needed. Such research could follow the work of Sinicato et al. [39], who compared serum cytokine levels with body fat content and other obesity markers. Thus, IL-39 may offer a more detailed assessment of the link between cytokines and BMI.

3.2. IL-39 serum levels

The mean study of serum IL-39 levels (Table 4) in patients with SLE was raised compared to those in the control group, i.e., 13.70 ± 0.35 ng/l vs. 10.67 ± 0.19 ng/l (P = 0.002).

Table 4: Mean distributions of IL-39 levels amongst SLE patients and controls

IL-39 (ng /l)				
Studied groups	**N**	**Mean**	**Std. Error**	***P* - Value**
Controls	**33**	**10.67**	**0.19**	**0.002**
Patients	**99**	**13.70**	**0.35**	

Highly significant differences (P = 0.004) were observed in the majority of statistical tests, i.e., ANOVA and LSD tests, with the exception of the comparison between the control group and patients with an inactive disease status (P = 0.45).
The mean serum IL-39 levels were highest in patients with active severe SLE, i.e., 17.42 ± 0.48 ng/l, and then decreased incrementally with lessening disease status, i.e., active moderate, 13.34 ± 0.23 ng/l; inactive, 10.93 ± 0.24 ng/l. The value for the control group was still lower, i.e., 10.67 ± 0.19 ng/l (Table 5).

Table 5: Mean distributions of IL-39 levels within SLE patient groups and controls

IL-39 (ng /l)				
Severity of SLE	**Mean**	**Std. Error**	**LSD test (*P* – Value)**	
Control	**10.67**	**0.19**	**A**	**0.45**
Inactive	**10.93**	**0.24**	**B**	**0.01**
Active moderate	**13.34**	**0.23**	**C**	**0.001**
Active severe	**17.42**	**0.48**	**D**	**0.01**
ANOVA test (P – Value); *P* = 0.004			**E**	**0.003**
			F	**0.01**

No difference was seen in mean IL-39 levels between patients who were or were not taking DMARDs: yes, 13.44 ±0.40 ng/l; no, 14.93 ± 0.68 ng/l (P = 0.11). A highly significant difference was seen with respect to mean IL-39 levels in patients who were or were not on steroids: yes, 12.91 ± 0.32 ng/l; no, 15.20 ± 0.77 ng/l (P = 0.002). Mean IL-39 levels also varied between patients who were or were not receiving biologics: yes, 17.16 ± 0.65 ng/l; no, 14.95 ± 0.41 ng/l (P = 0.02), as shown in Table 6.

Table 6: Mean distributions of IL-39 levels amongst patient groups in relation to treatment intake

IL-39 (ng /l)					
Treatment intake		**N**	**Mean**	**Std. Error**	***P* - Value**
DMARDs	**Yes**	**82**	**13.44**	**0.40**	**0.11**
	No	**17**	**14.93**	**0.68**	
	Total	**99**			
Steroid	**Yes**	**63**	**12.91**	**0.32**	**0.002**
	No	**36**	**15.20**	**0.77**	
	Total	**99**			
Biologics	**Yes**	**13**	**17.16**	**0.65**	**0.02**
	No	**53**	**14.95**	**0.41**	
	Total	**66**			

Correlation studies between IL-39 levels and other parameters in SLE patients demonstrated significant inverse relationships between serum IL-39 titres and C3 concentrations (r = -0.29, *P* = 0.004) and between serum IL-39 levels and C4 levels (r = -0.44, *P* = 0.0005).
Significant positive correlations were observed between serum IL-39 levels and ESR values (r = 0.35, *P* = 0.0007) and between serum IL-39 levels and anti-dsDNA antibody concentrations (r = 0.35, *P* = 0.01).
Additional weak positive or negative relationships were identified that failed to reach statistical significance.

Table 7: Correlations between IL-39 levels and additional SLE patient parameters

SLE patients (N = 99)		
Pearson Correlation		**IL-39 (ng/l)**
BMI	**r**	**0.06**
	P - Value	**0.53**
Age	**r**	**-0.003**
	P - Value	**0.97**
Duration	**r**	**0.11**
	P - Value	**0.29**
ESR	**r**	**0.35**
	P - Value	**0.0007**
Anti -ds DNA	**r**	**0.26**
	P - Value	**0.01**
C3	**r**	**-0.29**
	P - Value	**0.004**
C4	**r**	**-0.44**
	P - Value	**0.0005**

Although studies of IL-39 in humans are limited, research conducted with alternative cytokines and in other therapeutic areas may be insightful. For instance, Qiu et al. [40] measured the concentration of cytokines from the IL-12 family and found a positive correlation with anti-dsDNA antibody titres. When anti-inflammatory drugs were given to newly diagnosed SLE patients, their expression went down. This shows that these cytokines are linked to inflammatory processes and the development of SLE. Elevated IL-39 levels have also been detected in patients with rheumatoid arthritis (RA), highlighting the use of this cytokine for assessing disease activity [41, 42]. In the same way, the sera of SLE patients had higher levels of IL-39 than those of healthy controls, and there was a positive correlation between IL-39 and anti-dsDNA antibody titres.

Reynolds et al. [43] compared serum cytokine levels with established clinical biomarkers and demonstrated their potential use as biomarkers for SLE disease activity. These authors also demonstrated increased sensitivity and specificity when using cytokine measurements for monitoring SLE disease activity. By comparing IL-39 levels with typical diagnostic parameters, the validity of IL-39 as a biomarker can be substantiated further.

A positive correlation was found between IL-39 and ESR levels, as well as with anti-dsDNA antibody titres. The potential sensitivity and accuracy of tests using IL-39 levels for diagnosis and disease management tools are highlighted in Figure 1. However, the specificity of IL-39 was only 51.5%, which is poor and well below the threshold for clinical use. Since SLE is a heterogeneous condition, this problem could be fixed by comparing how the disease shows up in different people. Some cytokines have been linked to certain disease presentations [10, 44]. For instance, Reynolds et al. [43] demonstrated links between cytokines and specific clinical phenotypes and highlighted the value of cytokine profile assessments. So, finding links between IL-39 and specific clinical symptoms could make IL-39 a better diagnostic tool and help us learn more about SLE heterogeneity [10].

Al Ghuraibawi et al. [45] found no significant correlations between patient treatments and IL-39 levels. Despite these findings, their studies suggested that IL-39 played an anti-inflammatory role in RA, which the higher levels of IL-39 found in the biological treatment group may support. While overall, biological therapies target inflammation, they have diverse functions with varying targets [46]. Thus, understanding how IL-39 levels change according to the different biological therapies could offer further elucidation of this issue. These studies also demonstrated a trend towards lower IL-39 levels in patients taking steroids and DMARDS, although they failed to reach statistical significance. The varying results and lack of definitive data therefore highlight the need for more detailed research and prevent the implications of these findings from being fully ascertained.

Ruchakorn et al. [44] correlated disease severity and serum cytokine levels in patients with SLE and demonstrated their ability to predict and identify SLE patients at greatest risk. Similarly, within the current work, a comparison of data from individual SLE patient groups shows that the highest IL-39 levels were evident in the active severe SLE patient cohort, and the lowest titres were observed in the inactive and active moderate patient groups. These data suggest a correlation between higher IL-39 levels and increasing disease severity, thereby highlighting the cytokine's potential for disease monitoring and classification.
The increased use of steroids and biologics was associated with worsening disease severity. A more intense therapeutic regime is associated with various undesirable side effects, particularly in SLE [47]. Thus, the incorporation of IL-39 measurements as an adjunct to current diagnostic tests may improve disease management, enable earlier disease diagnosis, and consequently improve disease control [48, 49].

3.3. ROC curve analysis:

A validation of serum IL-39 tests was performed. Serum IL-39 levels exhibited good validity for the monitoring of patients with SLE when a cut-off value of ≥10.25 ng/l was employed. The obtained diagnostic performance parameters were: area under the curve (AUC), 0.79; sensitivity, 79.8%; specificity, 51.5%; positive predictive value (PPV), 82.3%; negative predictive value (NPV), 44.4%; accuracy, 71.97%, with a highly significant difference ($P = 0.001$, $P<0.01$) as shown in Figure 1.

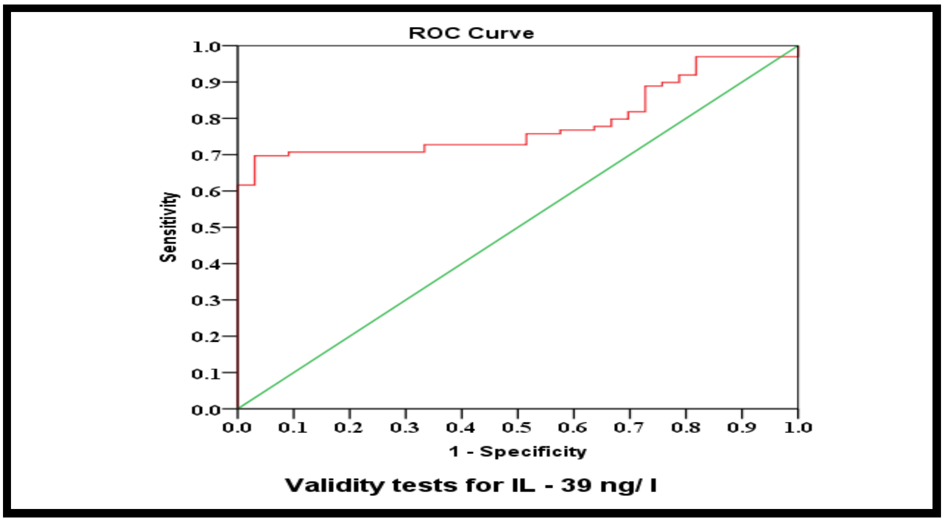

Figure 1: Validity tests relating to IL-39 levels obtained from operating characteristic curve analysis

4. Conclusion

The findings reported in other studies can be combined with the results of the current research in order to generate a clearer understanding of IL-39 and its use in the diagnosis of SLE. Patients with SLE had higher levels of IL-39 in their blood than healthy controls, and IL-39 levels were linked to the severity of SLE and the levels of typical clinical biomarkers. Thus, IL-39 may be a promising biomarker for monitoring SLE disease activity. More research is needed to find out what this cytokine does, where it goes, and how it contributes to immunopathogenic mechanisms. This could help with disease diagnosis, treatment, and management.

Ethical clearance

This study was approved by the Scientific Ethics Committee of the College of Medicine, University of Baghdad. Both the Ministry of Higher Education and Scientific Research and the Ministry of Health in Iraq approved it. The ethical approval number is 022.

Conflict of interest

The authors declare that they have no conflicts of interest.

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