Serum interleukin-40: an innovative diagnostic biomarker for patients with systemic lupus erythematosus

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ABSTRACT

Introduction: Interleukin (IL)-40 is a recently identified cytokine with a novel role in the pathogenesis of inflammatory diseases. Since systemic lupus erythematosus (SLE) is an autoimmune disease characterised by a pro-inflammatory response, it is likely that IL-40 contributes to the underlying disease processes of this disorder. The aim of the current study was to evaluate the potential of IL-40 to act as a diagnostic biomarker for SLE.

Matrrials and methods: The study included 99 patients with SLE who attended the Rheumatology Unit at Baghdad Teaching Hospital. These subjects were divided into three subgroups according to disease status: inactive, n = 33; active moderate, n = 33; and active severe, n = 33. Additionally, 33 matched controls were studied. Full medical histories, body mass index, gender and clinical disease activity, the latter evaluated with the SLE disease activity index, were collected. Laboratory parameters measured included anti-dsDNA antibodies, C3 and C4 levels, erythrocyte sedimentation rate and C-reactive protein titres. Serum IL-40 levels were quantified using an enzyme-linked immunosorbent assay.

Results: IL-40 levels were significantly higher in patients (12.5420 ± 3.00575 ng/L) than in controls (6.1138 ± 0.59452 ng/L; p < 0.01). Mean serum IL-40 concentration was highest in the active severe group (15.2291 ± 2.26540 ng/L) and decreased, in order of disease severity, in the remaining cohorts: active moderate, 13.0643 ± 1.23927 ng/L; inactive, 9.3325 ± 1.62807 ng/L (P < 0.01); controls, 6.1138 ± 0.59452 ng/L. Serum IL-40 levels showed excellent validity for the diagnosis of SLE with a cut-off value of ≥ 9.3 ng/ml and area under the curve of 0.987. Sensitivity, specificity and accuracy were 99%, 90.9% and 96.97%, respectively (P < 0.001).

Conclusions: Serum IL-40 levels were elevated in SLE patients. It is therefore proposed that IL-40 is a novel cytokine which is associated with SLE and positively linked with disease severity.

KEYWORDS:
Systemic lupus erythematosus; SLE; IL-40; autoimmune condition; inflammation

INTRODUCTION

Systemic lupus erythematosus (SLE) is a condition that develops owing to the abnormal immune-mediated destruction of healthy tissues[1,2] caused by B and T-cell hyperactivity and coincides with reactivity to self-antigens.[3] Increased production of antibodies, defective antibody clearance and complement and cytokine stimulation are some of the typical characteristics that result in the symptoms of SLE. There is up to a 3-fold increase in mortality in patients living with SLE compared to the general population. Improved treatment options may reduce mortality rates; however, superior diagnostic methods which allow for earlier or more sensitive detection of the disease are also essential.

The earliest signs of SLE reflect constitutional symptoms, which may be accompanied by mild to moderate joint pain, suggestive of arthritis. However, the presence of an accompanying skin rash or skin lesions at various anatomical sites supports a diagnosis of SLE.[1]

Since SLE is a heterogenous condition, to date, establishing a diagnosis has proved difficult as the presentation often reflects the symptoms of alternative conditions, e.g., cancers or infectious diseases, such as human immunodeficiency virus and acquired immunodeficiency syndrome.[3] Viral serological tests and tissue histopathological testing may be performed to exclude other causes. The American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) have proposed criteria for the diagnosis of SLE. However, as patients with mild diseases are commonly unrecognised by this classification, more rigorous testing is essential.[3]

Current diagnostic methods for the diagnosis of SLE rely on clinical symptom manifestations and are complemented by laboratory tests, such as viral or tissue investigations. The latter includes the anti-nuclear antibody (ANA) test; a positive ANA result is supported by an antigen-specific ANA for extractable nuclear antigens, which has a specificity of approximately 66% for these complexes. It is recommended that consultants collaborate with a SLE rheumatologist in order to attain a more reliable diagnosis. Only a few biomarkers have been recognised as being of value in the diagnosis of SLE, but as none of these can be utilised with confidence in disease management, novel biomarkers are urgently required in the field. Elucidation of more precise biomarkers for SLE could greatly improve detection sensitivity and reduce the time taken to diagnose patients. However, there has been little success to date.

This article was accepted: 21 July 2023
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Previous attempts to elucidate cytokine profiling in SLE have included the analysis of tumour necrosis factor-α (TNF-α), a pro-inflammatory cytokine which evidences increased expression in a variety of autoimmune diseases.[4] In one study, no differences between serum TNF-α levels in healthy and SLE groups were determined, although another study suggested that TNF-α was a useful biomarker for SLE. Thus, at present, the role of TNF-α in the diagnosis of SLE is unclear.[5]

A potentially superior approach is to quantify the levels of cytokines secreted by B cells, since SLE is predominantly mediated by aberrant B-cell activity, with autoimmune diseases, such as SLE, characterised by the presence of autoantibodies. At least one study has demonstrated aberrant B-cell-associated cytokine profiles in which IL-4 was virtually undetectable in the serum of SLE patients and coincided with a rise in IL-6.[6] The recent discovery of the B-cell-associated cytokine, IL-40, may also be utilised to improve the diagnosis of SLE, as exemplified in other inflammatory conditions.

IL-40 is a B-cell-associated orphan cytokine encoded by the gene, C17orf99, which is secreted via activated B-cells. This gene regulates IgG production in order to maintain the physiological function of B-cells.[7] Studies have demonstrated that IL-40 accumulates in the synovial joints of patients with rheumatoid arthritis (RA); serum IL-40 concentrations in patients with RA are substantially increased compared to those detected in healthy controls.[8] In RA, IL-40 propagates pro-inflammatory cytokine release and autoantibody production; extracellular IL-40 enhances the synthesis of tissue-degrading enzymes.[9] In one study, the depletion of B-cells reduced IL-40 production by 70%, suggesting that B-cell targeted therapies may offer relief from autoimmune conditions mediated by IL-40. However, other immune cells may synthesise residual IL-40. IL-40 has also been suggested to be a useful biomarker for the detection of type II diabetes mellitus and Sjögren's syndrome, underscoring its role in the pathogenesis of inflammatory and autoimmune diseases.[10-12]

Since SLE is an autoimmune condition characterised by a pro-inflammatory response, the detection of IL-40 may be a useful strategy for the identification of individuals with SLE as aberrant B-cell activity is a hallmark of the disease. However, conflicting evidence suggests that IL-40 only regulates local inflammation and does not underlie the systemic inflammatory response observed in patients with SLE in whom IL-40 levels were comparable to those measured in controls.10 Before the utility of IL-40 in the diagnosis of SLE can be realised, further studies are required in order to investigate whether IL-40 plays a role in SLE or whether it is simply a local inflammatory mediator.

The principal objective of this present study was to thoroughly evaluate and ascertain the potential efficacy of IL-40 as a reliable diagnostic biomarker for SLE.

MATERIALS AND METHODS

This study included 99 patients, aged over 18 years, who were diagnosed with SLE according to the 2019 EULAR/ACR classification criteria.[13] They were divided into three subgroups: inactive, *n*=33; active moderate, *n*=33; and active severe, *n*=33. Thirty-three age- and sex-matched healthy controls were also included. Participants were recruited between November 2022 and January 2023 from the Rheumatology Unit at the Baghdad Teaching Hospital. Exclusion criteria were: concurrent overlapping inflammatory arthritis, connective tissue disease or seronegative spondyloarthritis; malignancy; pregnancy; evidence of infection and patient refusal. Under the direction of the rheumatologist, the full patient information page data and consent form were completed, and the Committee of Scientific Ethics from the College of Medicine, University of Baghdad approved the study. The ethics committee's approval number was 023. For each patient, gathered baseline data encompassed blood investigations, full medical histories, body mass index (BMI), gender and clinical disease activity as evaluated with the SLE disease activity index. Disease-related laboratory parameters included anti-dsDNA antibodies, C3 and C4 levels, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

The SLE disease activity scoring system consists of 24 variables which cover 9 organ systems and yield a total score of 105. A total score ≤ 3 suggests that no flare is present, a total score > 3 and ≤ 12 is considered to reflect a mild to moderate flare, and a total score > 12 represents a severe flare.[14] Serum was obtained by centrifuging blood specimens for 10 to 15 minutes at 1000–3000 rpm. Serum samples were then frozen at –20°C. The enzyme-linked immunosorbent assay technique (Sun Long Biotech Company, China) was used to measure serum IL-40 in keeping with the manufacturer's instructions. A plate reader was used to determine the absorbance at 450 nm. The immunological testing was done at the International Centre for Research and Development.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 21 (IBM). Student's t tests, analysis of variance (ANOVA) and the less significant difference (LSD) test were performed for comparisons of quantitative variables, i.e. age, BMI and serum IL–40 levels, between studied groups. Normally distributed data are expressed as mean ± standard deviation. Pearson's chi-square test (χ^2) was used for comparisons of qualitative variables between studied groups, i.e. age groups and BMI. A binomial Z-test was performed for a comparison of gender and treatment intake. Pearson's correlation test was applied in order to detect the relationships between serum IL–40 levels and age, BMI, duration of SLE disease, ESR and C3 and C4 concentrations. The validity of the ELISA test was estimated with a ROC curve, cut-off value, area under curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. The statistical significance threshold was deemed to be a P-value < 0.05.

RESULTS

The age ranges of the 99 SLE patients and 33 control subjects were 18 to 58 years and 19 to 55 years, respectively. Table I illustrate the similarities between the two cohorts with respect to the demographic parameters of gender, age group and BMI.

Females were predominant within both studied groups, comprising 93 (93.94%) SLE patients and 30 (90.4%) controls (p=0.037).

The frequency of subjects was highest within the age range, 31–40 years, in both controls (13, 39.4%) and SLE patients (40, 40.4%), followed by the age range 18–30 years, i.e. controls (12, 36.4%) and SLE patients (36, 36.4%) (p=0.991). The mean ages of the two studied groups were similar, i.e. controls, 35.35 ± 11.783 years, and SLE patients, 34.69 ± 9.074 years (p=0.789).

The frequency of subjects assigned to the BMI classifications in the two cohorts was as follows: overweight: controls, 19 (57.6%), SLE patients, 40 (40.4%); obese: controls, 8 (24.2%), SLE patients, 34 (34.3%); normal weight: controls, 6 (18.2%), SLE patients, 25 (25.3%) (p=0.359).

Mean BMI showed a trend towards being greater in the SLE patient cohort when compared to the control group, i.e. 28.1342 ± 5.5956 kg/m^2 and 25.8926 ± 3.87481 kg/m2, respectively, but this failed to reach statistical significance (p=0.161).

When the variables were compared with respect to disease activity using ANOVA, no differences were identified (Table II). The mean BMI values of the SLE patients within all three groups of disease activity were similar: active severe, 28.7406 ± 6.27527 kg/m^2; active moderate, 28.3613 ± 6.04078 kg/m^2; inactive 27.8319 ± 4.66821 kg/m^2 (p=0.101).

LSD test values were also similar between the various levels of disease activity: inactive vs. active moderate, p=0.691; inactive vs. active severe, p=0.494; active moderate vs. active severe, p=0.775.

Mean disease durations were similar between the different disease activity groups: active moderate, 6.836 ± 5.3956 years; active severe, 5.994 ± 4.2940 years; inactive, 4.306 ± 4.7466 years (p=0.185). A within-group comparison of the LSD test data demonstrated no differences: inactive vs. active moderate, p=0.072; inactive vs. active severe, p=0.228; active moderate vs. active severe, p=0.546.

Mean serum ESR values were higher, the greater the disease activity: active severe disease, 47.18 ± 30.304; active moderate, 40.79 ± 26.415; inactive disease, 21.70 ± 11.509 (p<0.001). Similar results were obtained for the LSD test, with the exception of active moderate vs. active severe disease states (p=0.221).

Mean anti-dsDNA levels were modestly elevated in SLE patients with active severe disease (92.812 ± 143.821) when compared with those with active moderate disease (30.297 ± 24.2616); anti-dsDNA titres were decreased in patients with inactive disease (19.233 ± 3.7611, p=0.00).

Within-group comparisons were shown to be identical by the LSD test, with the exception of inactive vs. active moderate (p=0.539).

Mean C3 levels were lower in SLE patients within the active severe cohort (0.5858 ± 0.37691) compared to those with active moderate disease (0.6973 ± 0.39807) and increased in the inactive group (1.0548 ± 0.49356, p<0.001).

Significant differences (P < 0.01) were noted following the LSD test, with the exception of active moderate vs. active severe (p=0.253).

Mean C4 levels were diminished in SLE patients with active severe (0.0543 ± 0.05139) compared to those with active moderate (0.2642 ± 0.21645) and inactive disease (0.2812 ± 0.08521); these differences were significant (p<0.01) for all comparisons apart from inactive vs. active moderate (p=0.528).

Table III presents the distribution of the CRP data and treatment intake according to the severity of SLE disease. This was non-significant (p=0.164) for DMARDs intake: inactive: yes, 31 (93.9%), no, 2 (6.1%); active moderate: yes, 26 (78.8%), no, 7 (21.2%); active severe: yes, 25 (75.8%), no, 8 (24.2%).

The data showed a significant difference (p=0.033) for CRP: inactive: positive, 1 (3.03%) negative, 32 (96.97%); active moderate: positive, 2 (6.06%), negative, 31 (93.94%); active severe: positive, 6 (18.18%), negative, 27 (81.82%).

Significant differences (p<0.01) were observed for other types of treatment intakes: (i) steroid intake: inactive: yes, 12 (36.4%), no, 21 (63.6%); active moderate: yes, 25 (75.8%), no, 8 (24.2%); active severe: yes, 28 (84.85%), no, 5 (15.15%) (P < 0.001); and (ii) biologics intake: active moderate: yes, 1 (3%), no, 32 (97%); active severe, yes, 12 (36.4%), no, 21 (63.6%) (p=0.008).

Result indicates that the mean serum IL-40 ng/ml titre in SLE patients (n=99) was higher than in controls (n=33), i.e. 12.5420 ± 3.00575 ng/L vs. 6.1138 ± 0.59452 ng/L (p<0.01).

It can be clearly observed from the ANOVA and LSD tests presented in Table IV that the mean IL-40 levels in the sera of SLE patients in the active severe cohort are higher (15.2291 ± 2.26540 ng/L) than in those patients in the active moderate group (13.0643 ± 1.23927 ng/L). The latter values are elevated compared to the inactive (9.3325 ± 1.62807 ng/L) and control groups (6.1138 ± 0.59452 ng/L) (p<0.01 in all cases).

Results also show the mean distributions of IL-40 levels in the sera of SLE patients according to the type of treatment intake. For DMARDs intake, mean IL-40 values were lower in those patients taking this medication: Yes, 12.2198 ± 2.96423 ng/L; No, 14.0959 ± 2.78502 ng/L (p=0.018). Mean IL-40 levels were similar amongst patients who were or were not on steroid therapy: Yes, 12.2301 ± 2.72639 ng/L; No, 13.1383 ± 3.44324 ng/L (p=0.154), and between patients who were or were not receiving biologics: Yes, 14.2086 ± 2.74526 ng/L; No, 14.1315 ± 1.96211 ng/L (p=0.907).

A correlation study between IL-40 levels and the other SLE patient parameters revealed that there were inverse

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

Parameters		Studied groups		*p* value
		Controls (n=33)	Patients (n=99)	
Gender	Male	3 (9.1%)	6 (6.06%)	0.337
	Female	30 (90.9%)	93 (93.94%)	NS
Age-groups (years)	18–30	12 (36.4%)	36 (36.4%)	0.991
	31–40	13 (39.4%)	40 (40.4%)	NS
	41–50	7 (21.2%)	21 (21.2%)	
	51–60	1 (3%)	2 (2%)	
BMI groups	Normal weight	6 (18.2%)	25 (25.3%)	0.359
	Overweight	19 (57.6%)	40 (40.4%)	
	Obese	8 (24.2%)	34 (34.3%)	
Age (years)	Mean	35.35	34.69	0.786 NS
	Std. deviation	11.783	9.074	
	Std. error	1.915	1.074	
BMI (kg/m^2)	Mean	25.8926	28.1342	0.161 NS
	Std. deviation	3.87481	5.5956	
	Std. error	0.63942	0.5483	

NS: non-significant (p>0.05), BMI: body mass index.

Table II: Mean distributions of parameters within SLE patient groups

SLE patient groups		Mean	Std. Deviation	Std. Error	LSD test (p value)	
BMI	Inactive	27.8319	4.66821	0.81263	A	0.691
	Active moderate	28.3613	6.04078	1.05156	B	0.494
	Active severe	28.7406	6.27527	1.09238	C	0.775
	ANOVA test (p value):		p=0.101			
Duration (years)	Inactive	4.306	4.7466	0.8263	A	0.072
	Active moderate	6.836	7.3956	1.2874	B	0.228
	Active severe	5.994	4.2940	0.7475	C	0.546
	ANOVA test (p value):		p=0.185			
ESR	Inactive	21.70	11.509	2.004	A	0.00
	Active moderate	40.79	26.415	4.598	B	0.00
	Active severe	47.18	30.304	5.275	C	0.221
	ANOVA test (p value):		p<0.001			
Anti-dsDNA	Inactive	19.233	3.7611	0.6547	A	0.539
	Active moderate	30.297	24.2616	4.2234	B	0.00
	Active severe	92.812	143.821	25.0362	C	0.001
	ANOVA test (p value):		p<0.001			
C3	Inactive	1.0548	0.49356	0.08592	A	0.00
	Active moderate	0.6973	0.39807	0.06929	B	0.00
	Active severe	0.5858	0.37691	0.06561	C	0.253
	ANOVA test (p value):		p<0.001			
C4	Inactive	0.2812	0.08521	0.01483	A	0.582
	Active moderate	0.2642	0.21645	0.03768	B	0.00
	Active severe	0.0543	0.05139	0.00895	C	0.00
	ANOVA test (p value):		p<0.001			

BMI: body mass index, ESR: erythrocyte sedimentation rate, Anti-ds DNA: Anti-double stranded DNA, C3 and C4: Complement components 3 and 4, LSD: Least Significant Difference, A = inactive vs. active moderate, B = inactive vs. active severe, C = active moderate vs. active severe.

Table III: C-reactive protein values and treatment intake distributions within SLE patient groups

Parameters		SLE patient groups			*p* value
		Inactive (n=33)	Active moderate (n=33)	Active severe (n=33)	
CRP	Positive	1 (3.03%)	2 (6.06%)	6 (18.18%)	**0.033
	Negative	32 (96.97%)	31 (93.94%)	27 (81.82%)	
DMARDs intake	Yes	31 (93.9%)	26 (78.8%)	25 (75.8%)	0.164 NS
	No	2 (6.1%)	7 (21.2%)	8 (24.2%)	
Steroid intake	Yes	12 (36.4%)	25 (75.8%)	28 (84.85%)	**<0.01
	No	21 (63.6%)	8 (24.2%)	5 (15.15%)	
Biologics intake	Yes		1 (3%)	12 (36.4%)	**0.008
	No		32 (97%)	21 (63.6%)	

**p<0.01. NS: non-significant (p>0.05), CRP: C-Reactive Protein, DMARDs: Disease- Modifying Anti-Rheumatic Drugs

Table IV: Mean distributions of IL-40 levels within SLE patient groups and controls

	IL-40 levels (ng/L)				
Severity of SLE	**Mean**	**SD**	**Std. error**	**LSD test (P value)**	
Control	6.1138	0.59452	0.07475	A	**<0.01
Inactive	9.3325	1.62807	0.28341	B	**<0.01
Active moderate	13.0643	1.23927	0.21573	C	**<0.01
Active severe	15.2291	2.26540	0.39436	D	**<0.01
ANOVA test (p value); p=0.00				E	**<0.01
				F	**<0.01

**(p< 0.01), SD: Standard Deviation, LSD: Least Significant Difference

Table V: Correlation study between IL-40 levels and other SLE patient parameters

	SLE patients (n = 99)	
Pearson Correlation		**IL40 ng/L**
BMI	r	0.069
	p value	0.495
Age	r	0.139
	p value	0.171
Duration	r	0.181
	p value	0.073
ESR	r	0.344
	p value	0.00
Anti-dsDNA	r	0.166
	p value	0.101
C3	r	-0.420
	p value	0.00
C4	r	-0.396
	p value	0.00

relationships between serum IL-40 titres and C3 (r = –0.420, p<0.01) and C4 levels (r=–0.396, p<0.01), and a positive relationship between serum IL-40 levels and ESR values (r=0.344, p<0.01).

The remaining variables demonstrated a weakly positive correlation which was insignificant (Table V).

Validity of Tests

The results given prove that serum IL-40 levels have excellent validity for use in the diagnosis or follow-up of SLE patients at a cut-off value of 9.3 ng/ml. The performance parameters were: AUC, 0.987; sensitivity, 99%; specificity, 90.9%; PPV, 97%; NPV, 96.8%; accuracy, 96.97% (p<0.001).

DISCUSSION

It is well-established that SLE arises from a complicated, multifactorial interaction between various genetic factors. Multiple genes contribute towards patient disease susceptibility, which is further refined and controlled by environmental triggers.[15-18] Research has been ongoing for several decades in order to identify relevant biomarkers for SLE,[19-22] and although some have shown promising results, no single biomarker has been able to detect SLE completely and reliably in every case. This issue arises as a result of the heterogeneous characteristics of SLE, the differing symptom presentations observed in practice,[23] and the complex patterns of hereditability and genetic variation associated with the disease.[24]

Nonetheless, previous research has indicated that IL-40 plays a central role in biological disease processes,[7-8,10,25] and the cytokine has recently been proposed as a contributing factor to the development of SLE-associated nephritis.[26] In this study, multiple blood markers were evaluated, including IL-40 titres, in order to investigate whether or not they played a role in the development and expression of SLE in the selected study population.

It was established that serum IL-40 concentrations differed significantly between patients with SLE and the controls. A positive association between IL-40 levels and lupus severity was identified, in that serum IL-40 titres increased in parallel with the severity and duration of lupus symptoms. Previous studies which have analysed serum IL-40 levels have shown a similar trend in relation to the identification of RA and concluded that IL-40 is a reliable indicator for the disease 25. IL-40 is a cytokine that plays a central role in the regulation and secretion of IgG which, in turn, supports the normal functioning of B cells and enables the body's immune system to effectively respond to antibodies.[7-8,27] IL-40 has not been widely studied, but it appears to be expressed only in mammals. It has a unique structure, which makes it incomparable to most cytokine families.[7,8] The cytokine has been shown to exert its most potent regulatory influence over B cells, acting during foetal development, and within the liver and bone marrow.[7,28-29] Studies of IL-40 knockout mice demonstrated an effect on B cell development, resulting in impaired and non-functioning cells.[8,25] Given that SLE is recognised as an autoimmune disease,[6,25] the current results support the hypothesis that IL-40 could be used to determine and diagnose autoimmune dysfunction.

The role of IL-40 and its efficacy as a biomarker for detecting disease have now been identified for a range of pathologies

including RA[8,25] type II diabetes,[10] hepatocellular carcinoma[30] and lupus.[11] The present results also imply that IL-40 is an important biomarker for the detection of SLE, and it is suggested that further work should be targeted towards the part played by IL-40 in disease development processes. Monitoring serum IL-40 levels could support future clinical diagnosis. Additionally, higher IL-40 titres were associated with SLE symptom severity and so serum IL-40 level monitoring in individuals suspected of having SLE could provide opportunities for early diagnosis and intervention.

It was determined that the complement indicators, C3 and C4, were both negatively correlated with IL-40 levels, indicating that a higher titre of IL-40, which is indicative of a positive SLE diagnosis, is associated with reduced C3 and C4 levels. This finding supports previous studies which have reported diminished blood serum complement factors in cases of lupus.[9,11]

When compared to the cohort of patients with inactive SLE, ESR values were elevated in the groups with active severe and active moderate disease, which supports existing literature showing that a high ESR indicates active lupus.[19] Elevated anti-dsDNA antibody levels were also measured in the active severe group as opposed to in SLE patients with active moderate or inactive disease, a finding which supports previous studies that suggest a high level of serum anti-dsDNA antibodies are strongly associated with lupus.

CRP has a complex role in lupus, with modest CRP elevations often seen in patients with SLE. In the current study, the inactive group exhibited more negative than positive test outcomes. Conversely, both types of active SLE patient cohorts demonstrated more positive than negative test outcomes. The reason for the variation in proportions of positive and negative tests across the different SLE types is not clear. However, these findings support previous observations, i.e. that CRP involvement in lupus appears to be part of a complex set of processes.[19]

A negative association between the use of DMARDs and IL-40 levels was observed, i.e. patients who did not take DMARDs had significantly higher serum IL-40 levels. This suggested that DMARDs may provide a protective effect against rising IL-40 levels in cases of SLE.

This study is the first to demonstrate the positive association between IL-40 levels and SLE symptom severity, adding evidence to suggest that IL-40 plays a role in SLE, and could be used as part of the diagnostic process. A robust sample size of 99 SLE and 33 controls was studied. However, it was recognised that when filtering patients by certain variables, the sample size was reduced to a less than ideal number. For instance, the sample size for SLE patients not taking DMARDs was small, i.e. 17 patients, when compared to the sample of 82 patients who were taking DMARDs. Nonetheless, it is considered that the results offer robust evidence that DMARDs may be protective against the development of SLE and support the role of IL-40 in this autoimmune condition. Sample sizes will always be a challenge in such studies, but it is recommended that, where possible, future studies should take all available steps to maximise sample sizes.

The link between IL-40 titres, and SLE symptom severity and duration, needs to be confirmed in a larger sample size, and in patient populations with greater ethnic diversity. Previous studies have highlighted that Black, Hispanic and Asian populations demonstrate higher rates of SLE,[31,32] and it is well-established that SLE is linked to genetic heritage and ancestry.[24] Furthermore, women are disproportionately affected over men.[23] A logical next step would therefore be to understand how the role and function of IL-40 may differ between different ethnic and geo-spatially distinct populations of individuals with SLE. The majority of studies have been conducted in the Western or developed world,[14-15,31,32] and a need for further studies to investigate the prevalence and presence of SLE in developing nations is recognised. This is especially relevant given that White populations appear to be at a lower risk of the condition.[31,32]

CONCLUSIONS

It was established that serum IL-40 measurements demonstrated strong validity for the identification and diagnosis of SLE, and exhibited greater accuracy than recognised in relation to other disease indications. IL-40 levels were positively correlated with SLE symptom severity and duration, indicating that this cytokine could be a promising biomarker for SLE, and play a role in early diagnosis and intervention monitoring. This study adds further evidence to support the observation that IL-40 is important with respect to the immune response and immune system regulation. It is hoped that it inspires further studies which are designed to improve the understanding of the cytokine's potential as a biomarker for SLE and other autoimmune diseases.

CONFLICTS OF INTEREST

No conflicts of interest.

FUNDING

This research article is entirely funded by the authors.

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