Iraqi Journal of Market Research and Consumer Protection
(2023) 15(2): 31-38
DOI: http://dx.doi.org/10.28936/jmracpc15.2.2023.(4)
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المجلة العراقية لبحوث السوق وحماية المستهلك

ASSOCIATION OF IL-37 AND IL-40 WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN FEMALE IRAQI PATIENTS

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Received 8/ 3/ 2023, Accepted 10/ 5/ 2023, Published 31/ 12/ 2023

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ABSTRACT

The primary cause of systemic lupus erythematosus (SLE) is autoantibodies to self-antigens. Interleukin 37 (IL-37) and Interleukin 40 (IL-40) are important cytokines which may play a role in regulating inflammation and involved in pathogenesis of autoimmune diseases like SLE. This study was aimed the interleukin levels (IL-37 and IL-40) during systemic lupus erythematous (SLE) development in Iraqi female patients. The present study was conducted on 100 female SLE patients and 100 healthy female controls, with mean ages of patients and controls (32.85 ± 0.99 and 32.05 ± 0.91)years, respectively. Erythrocytes Sedimentation Rate (ESR) and C-reactive protein (CRP) were investigated in this study. Enzyme-linked immune sorbent assay(ELISA) kits examined levels of interleukins in the serum of patients and healthy controls. The results found that IL-37 levels in SLE patients were lower than in controls(23.39 ±0.92ng/mL *vs*48.02 ±0.46ng/mL), respectively, with a statistically significant. While serum IL-40 levels appeared to have a higher significant in patients compared with the healthy control (9.98 ±0.32ng/mL vs4.16 ±0.05ng/mL) respectively. Also, ESR and CRP was high significant in patients compared with healthy controls. In conclusion, these findings suggest that IL-40 and IL-37 could be used as a biomarker for SLE.

Keywords: autoimmunity, systemic lupus erythematosus, IL-37, IL-40.

علاقة الانترلوكينات 37 و40 مع داء الذئبة الحمامية الجهازية في نساء مرضى عراقيات

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الخلاصة

السبب الرئيسي لمرض الذئبة الحمامية الجهازية (SLE) هو الأجسام المضادة الذاتية للمستضدات الذاتية. انترلوكين 37و40 من السايتوكينات الهامة التي قد تلعب دورًا في تنظيم الالتهاب وتشارك في التسبب في أمراض المناعة الذاتية مثل مرض الذئبة الحمراء. في هذه الدراسة تم قياس مستويات الإنترلوكين (IL-37 و IL-40) أثناء تطور الذئبة الحمامية الجهازية (SLE) في مريضات عراقيات. أجريت الدراسة الحالية على مئة مريضة مصابة بمرض الذئبة الحمامية و مئة اخرى من الإناث الأصحاء، بمتوسط أعمار المرضى والاصحاء (32.85 ± 0.99 و 32.05 ± 0.91) سنة، على التوالي. تم فحص معدل ترسيب كريات الدم الحمراء (ESR) والبروتين (CRP) في هذه الدراسة. فحصت مجموعات الالايزا مستويات الإنترلوكينات في مصل المرضى والاصحاء. وجدت النتائج أن مستويات IL-37 في مرضى الذئبة الحمراء كانت أقل مما كانت عليه في االاصحاء (23.39 ± 0.92 ng / mL مقابل 48.02 ± 0.46 ng / mL)، على التوالي ، مع وجود فرق معنوي ((p = 0.0001)). بينما ظهر أن مستويات المصل (IL-40) لها فرق معنوي أعلى (p<0.0001) في المرضى مقارنة مع الاصحاء (9.98 ± 0.32 ng / mL مقابل 4.16 ± 0.05 ng / mL) على التوالي. اخيرا، تشير هذه النتائج إلى أنه يمكن استخدام IL-40 و IL-37 كمؤشر حيوي لمرض SLE.

الكلمات المفتاحية: المناعة الذاتية، الإنترلوكين، الذئبة الحمامية الجهازية، IL-37، IL-40.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease of multifactorial etiology with extensive multi-systemic effects. The most distinguishing signs and symptoms that make the disease easy to identify are lesions of skin (malar and discoid rash), in the nose, mouth manifestations of musculo skeletal like arthritis, fractures of bone fragility, and amplification of secondary pain **(Levy & Kamphuis, 2012)**. SLE is disorders of systemic autoimmune because of auto-antibodies such as antibodies against double stranded DNA ((anti-ds DNA)) and anti small nuclear RNA-binding protein. Anti ds-DNA antibodies (Abs) diagnose patients and study the disease's pathogenesis **(Isenberg *et al*,. 2007)**. Other biomarkers used in the diagnosis of SLE are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (**Egner, 2000**). Unfortunately, serologic test is no reliably and accurate disease activity measures (**Reveille, 2004**). Therefore, new specific biomarkers finding is important and there is obtain new of constant effort ones to manage the disease better. Cytokines are secreted proteins characteristic as small produced by immune system cells. Cytokines regulate immune and inflammatory responses. Studies shows that disturbances of expression of cytokines are SLE pathogenesis significant (**Lourenço & La, 2009**). It have been shown as a biomarker for lupus susceptibility, diagnosis, and regulation of immune response. This study focuses on two cytokines from the interleukin (IL)-1 familyIL37 and IL-40. They have immune modulatory actions and are a novel anti-inflammatory **(Alyaqubi *et al*., 2016)**. IL-37 was first described as an IL1 family member through analyses of bioinformatics of human genome for uncharacterized genes which share similarities and locations of chromosomal with other family members of known IL-1. IL37 gene of human is located at (2q14.1) chromosome **(El-Sayed *et al*., 2018**). Most of IL-37 effects in, autoimmune, infectious diseases and metabolic have been tested in autoimmune and several metabolic (**Nold *et al*., 2010**). The last cytokine discovered is IL-40. In October 2017 it Reported (**Catalan *et al*., 2017**). Encoding IL-40 gene is annotated in genome of human as C17orf 99. It is expressed by activated B cells, fetal liver, encoding a secreted small proteins (27kDa) of 265 amino acids. We became this gene interested while screening the of gene expression of body index (BIGE) data (**Roth *et al*., 2006**) for uncharacterized genes to immune system related. therefore, it is not related structurally to other family of cytokine, indicator that it similarly has a unique history of evolutionary. An estimated (10%) of genome the human encode secreted proteins, and C17orf 99 has been identified interest as a potential gene on a wide screen of uncharacterized genes which secreted (**Clark *et al*., 2003**).The current study aimed to measure the concentration of interleukins (37 and 40)in patients with SLE and compare them with control models.

MATERIALS AND METHODS

patients and controls

Subject

One hundred female patients with SLE with a mean age of (32.85 ±0.99 years) were included in this study; they were obtained from Baghdad city (medical city, Baghdad Hospital)/ Iraq and diagnosed SLE patients based on antinuclear antibody (ANA) and anti-double stranded DNA (anti-dsDNA) these tests were taken from patients archives, Erythrocyte Sedimentation Rate (ESR), serum C-reactive Protein (CRP). All the study participants' patients had written informed consent and the approval of the local ethics committee (CSEC/0122/0001). In this study the blood samples of 100 Female healthy controls were obtained from National Blood Transfusion Center.

Blood Collection

Five milliliters of blood were taken from every patient and control subject. The blood was placed into gel tubes to obtain serum. The serum was then clotted at 4°C for an hour and centrifuged at 2000g for 10 minutes to determine the level of interleukins.

Measurement of interleukins IL-37 and IL-40 levels

The obtained serum was stored at (–20°C) until analysis. Measurements of interleukins (IL-37 and IL-40)in the serum samples were performed using the enzyme-linked immune sorbent assay(ELISA) sandwich-kits (BT-Lab, China) by the manufacturer's protocols with catalog number (IL-37 Cat.No:SL2231Hu), (IL-40 Cat.No:SL3535Hu).

STATISTICAL ANALYSIS

The impact of various factors on the study parameters was determined using the Statistical Analysis System- SAS (**2018**) program. The T-test was employed to compare means significantly. A significant comparison between percentages (0.05 and 0.01 probability) was made using the chi-square test (**Cary, 2018**). The receiver operating characteristic (ROC) curve analysis was applied to calculate the area under the curve (AUC), the 95% confidence interval(CI), the cut-off value of (adjusted by the Youden Index), and the sensitivity and specificity.

RESULTS AND DISCUSSION

The study recruited 100 female SLE patients with a mean age of 32.85± 0.99 years old and 100 female controls with a mean age of 32.05 ± 0.91years old. ESR and C-reactive protein (CRP) are biomarkers to diagnose and monitor SLE**.** ESR concentration was 31.80 ± 2.02 mm/h. CRP was positive in 27 patients, with a percentage of 27.00%, and negative in 73 patients, with a percentage of 73.00%.

Table (1): Demographic and clinical features of control and patients

Parameters	**(mean± S.D.)**		***p*-value**	**Normal value**
	Control No.=100	Patients No.=100		
Ages(years)	32.05 ±0.91	32.85 ±0.99	0.556	-
ESR mm/h	9.00 ±0.36	31.80 ±2.02	0.0001**	0-20
CRP IU/ml	Negative(73.00%)	Positive(27.00%)	0.0006**	-

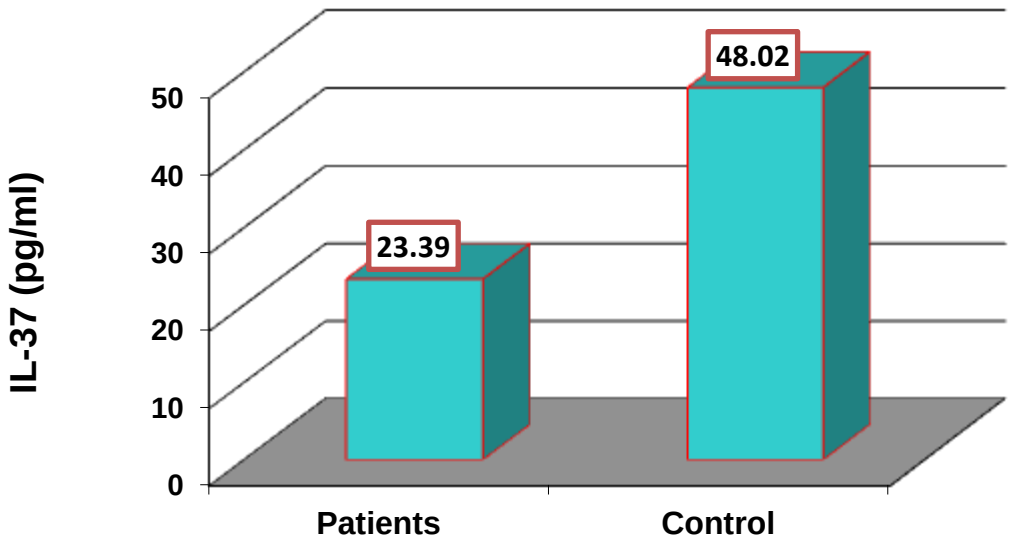
In our study, SLE patients had high ESR and CRP biomarkers levels, While the control was within the normal range, as shown in table (1). Studies by (**Serdaroğlu *et al.*, 2008**; **Vanichapuntu *et al.*, 2010**) supported this result. They demonstrated that these biomarkers had yet to enable them to monitor the activity of rheumatoid arthritis (RA) accurately. Also, our study agreed with (**Yao *et al.*, 2014**) which demonstrated that serum cytokines and levels of urinary were elevated of significantly and correlated with activity of disease, ESR, CRP and anti-dsDNA in SLE patients.

Serum levels of interleukins 37 and 40 in the study groups

The levels of IL-37 and IL-40, in both SLE patients and healthy controls, were estimated by using the ELISA technique, and the corresponding levels were (23.39 ±0.92(pg/ml)vs48.02 ±0.46(pg/ml)), (9.98 ±0.32pg/mL vs4.16 ±0.05pg/mL) respectively. The SLE group had significantly lower serum levels of IL-37 than healthy controls (P<0.001). At the same time, SLE patients had significantly greater serum levels of IL-40 than healthy controls(P<0.001). Table (2) and Figures1,2 shows the serum levels of IL-37 and IL-40 in SLE patients and healthy controls.

Table (2): Comparison between patients and control groups IL-37 and IL-40 with Systemic Lupus Erythematosus.

Group	Mean ± SE	
	IL-37(pg/ml)	IL-40(pg/ml)
Patients	23.39 ±0.92	9.98 ±0.32
Control	48.02 ±0.46	4.16 ±0.05
T-test	2.045 **	0.637 **
P-value	0.0001	0.0001
** (P≤0.01).		



Figure(1): Comparison between patients and control groups in IL-37 with SLE.

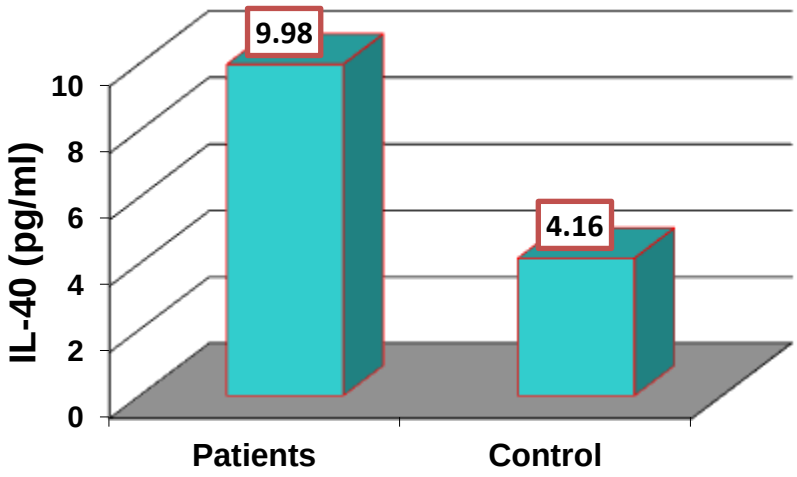


Figure (2): Comparison between patients and control groups with SLE in IL-40.

In immune system cells such as T-cells, IL37 is expression (**Pan *et al.*, 2020**). Its involved in the immune response and inflammatory process (**Rodriguez *et al.*, 2018**). In this study, interleukin (IL)-37 levels were observed to be reduced, and these results agreed with(**Wang *et al.*, 2018**), which suggested that IL37 may involve in the SLE pathogenesis by regulating IFN-γ, IL-18BP and IL-6. Also, this results agreed with (**Horwitz *et al.*, 1998**) suggest IL-12 serum level is decreased in patients with SLE recent onset compared with healthy controls. Also, the results agreed with (**Qian *et al.*, 2021**), which showed low levels of IL37 were found in patients of SLE with a discoid rash when patients compared who didnot have symptom. Also, levels of Plasma IL-37 were significant lower in hypocomplementemia patients than in those without the feature. In contrast to IL-40 cytokines, it is identified as a cytokine associated B-cell related to mechanisms of immune response and homeostasis of B-cell (**Catalan *et al.*, 2017**). Our results showed an increase in serum levels of IL-40, which agreed with (**Feng *et al.*, 2020**), which demonstrated that IL40 was increased in the serum paired samples and synovial fluid of R.A. compared of patients to osteoarthritis and healthy control. Also, these results agreed with (**Navrátilová *et al.*, 2021**), which showed increased IL40 in R.A sera cases compared to controls. The C17orf99 gene encodes IL-40. Its discovery in 2017, IL-40 of literature and its implication are limited in disease processes. Characterizing the encoded protein identified the C17orf99 gene as an autoimmune hepatitis autoantigen and provided of role the first evidence in autoimmune and anti-inflammatory (**Zingaretti *et al.*, 2012**).

Recivier Operating Characterstic (ROC) curve of interlukins

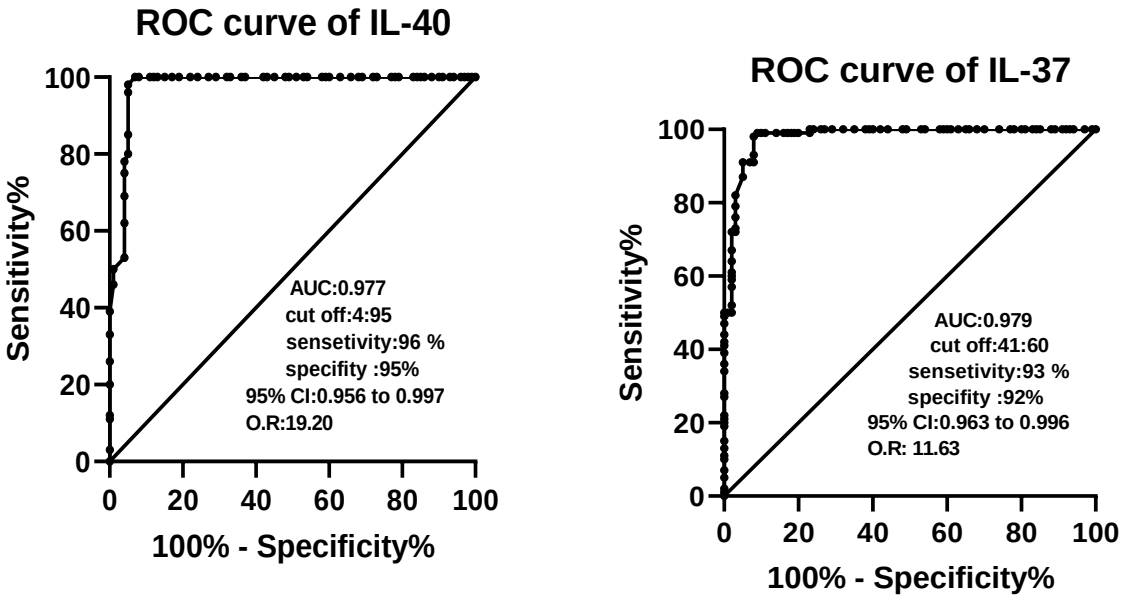
The receivers operating characteristics (ROC) curve Analysis confirmed the findings of interleukins results. IL-37 occupied a significant area under curve (AUC), which is 0.979 ($p <$ 0.0001). At a cut-off value of 41.60pg/mL, the specificity and sensitivity of IL-37 were 92.0% and 93.0%, respectively Figure (3A). The ROC curve in SLE patients revealed that IL-40 occupied a significant AUC of 0.977 ($p<0.0001$). At the cutoff value of 4.95pg/ml, the specificity and sensitivity were 95% and 96%, respectively. Figure (3B). The ROC curve and AUC were used for the diagnostic power evaluating of the IL-37 and IL-40 as possible

biomarkers. The ROC curve is the tool of popular graphical for the diagnostic power evaluating of a biomarker. It provides an exhaustive look at the sensitivity trend overall cutoff and thus present information about the association between the specificity and sensitivity of biomarkers. The AUC, which integrates the curve's overall cutoffs, for an efficient summarization is proposed (**Hsu *et al.*, 2014**) in SLE healthy controls versus patients showing probability(*p*) and (AUC). The AUC refers to the overall of a diagnostic marker performance to distinguish between cases without and with the disease or a condition of test. An AUC of (0.50–0.59) suggested no discrimination, (0.60–0.69) indicates poor discrimination, (0.70–0.79) is considered accepted, (0.80–0.89) is considered excellent, and (≥ 0.9) is outstanding (**Jaber & Ad'hiah, 2023**) Therefore, from the ROC results can use IL-37 and IL-40 as biomarkers in SLE disease.

Table (3): Sensitivity and specificity of IL-37 and IL-40 between all systemic lupus erythematosus (SLE) patients and controls.

parameter	Cut-off	AUC	Sens.(%)	Spec.(%)	95%CI	O.R	P
IL-37	41,6	0.97	93	92	0.96-0.99	11.6	< 0.0001
IL-40	4.95	0.97	96	95	0.95-0.99	19.2	< 0.0001

AUC: area under the curve; Sens. : sensitivity; Spec. : specificity, O.R: odds ratio. Bold values are significant at p < 0.05.



(B) (A)

Figure (3): Receiver operating characteristic (ROC) curve of (A) for IL37 and (B) for IL-40.

CONCLUSION

Serum levels of IL-37 were shown to be decreased in the serum of female SLE patients. While IL-40 serum level was increased in the female patients of SLE compared with controls. From results of AUC might consider these interleukins as biomarkers in SLE pathogenesis.

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