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Original article

Interleukin 36α Serum Level in Egyptian Patients with Systemic Lupus Erythematosus and its relation to Disease Activity

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Abstract

Background: Systemic lupus erythematosus (SLE) is a systemic illness marked by clinical variability, unpredictable outcome, and recurring flares. Interleukin 36α is crucial for innate immunity. It is strongly hypothesized that IL-36 has an impact on SLE pathogenesis. **Aim:** Assessing the link between interleukin 36α levels and disease activity in systemic lupus erythematosus (SLE). **Subjects and Methods:** This study examined forty systemic lupus erythematosus patients and 40 controls. The SLE disease activity index (SLEDAI) establishes disease activity. ELISA estimates serum Interleukin 36α. **Results:** Interleukin 36 α levels were considerably higher in SLE patients compared with the controls (P < 0.001). IL-36α levels significantly linked to the SLE disease activity index (SLEDAI) score (P <0.001), age (P = 0.002), disease duration (P = 0.00), arthritis (P <0.001), nephritis (P = 0.002), and anti-

double-stranded DNA antibody (P <0.001). IL-36 α levels and complement 3 were inversely linked (P < 0.001). **Conclusion:** Interleukin 36α may identify SLE activity early in follow-up.

1. Introduction:

SLE is a systemic illness marked by clinical variability, unpredictable outcome, and recurring flares. Rarely, it might be organ-dominant, causing diagnostic difficulties [1]. It develops a wide variety of autoantibodies and damages numerous organs [2].

The complicated pathogenesis of SLE is characterized by defective apoptosis, and autoantibody surplus production, causing inflammation and immunological complexes genesis [3]. The SLE disease activity index 2000 (SLEDAI-2K), C3 and C4 complement, and anti-dsDNA antibodies are utilized daily to evaluate SLE activity [4].

The cytokine IL36 belongs to the IL1 cytokine class, which is crucial for innate immunity. IL36 is found in epithelial cells, keratinocytes, monocytes/macrophages, and T lymphocytes [5]. A prior research found that IL-36 influences instances and intensity of psoriasis. [6].

Primary Sjogren's syndrome (pSS) patients reported greater IL-36 and disease activity [7].

Contemporary studies have shown a connection between IL-36 levels and SLE illness intensity. Despite high blood levels, however, there is variability in IL-36 expression in SLE individuals [8-9]. The purpose of our research was to analyze the interleukin 36 levels in SLE individuals and their link with SLE activity.

2. Patients and Methods:

A comparative case-control inquiry The Rheumatology and Rehabilitation Department of Beni-Suef University Hospital recruited 40 clinically confirmed SLE patients, according to the 2019 EULAR/ACR [10], between March and December 2021. Forty healthy gender- and age-balanced controls were included. 38 females (95.0%) and 2 males (5.0%) were ages 20–45, with a mean of 32.8±7.8. Controls were 19–43 years old, with a mean of 31±5.3 years.

Exclusion criteria:

1. Infection.
2. Cancer.
3. Severe organ failure.
4. Another autoimmune condition.

Concerns with ethics every participant gave their written consent before the trial. The Beni Suef University School of Medicine's Ethical Medical Committee gave this work their blessing.

Clinical assessment

All patients possessed a full medical history and physical examination to compile socio-demographic and clinical information such as age, gender, illness duration, systemic involvement, and drug therapies. SLEDAI-2K gauges SLE illness activity [11].

SLEDAI scores were used to categorize activity levels: no activity (SLEDAI = 0), mild activity (SLEDAI = 1-5), moderate activity (SLEDAI = 6-10), high activity (SLEDAI = 11-19), and very high activity (SLEDAI ≥ 20) [12].

Utilizing the Systemic Lupus International Collaborative Clinics/ACR (SLICC/ACR) damage index to figure out the severity of SLE illness [13]. The SCLICC/ACR damage index reflects cumulative end-organ damage in SLE. Damage is the irrevocable change that has occurred since lupus emergence and is unrelated to ongoing inflammation.

Routine laboratory assessment:

Samples of peripheral blood were obtained from all research participants. Complete blood count (CBC) and erythrocyte sedimentation rate (ESR) using the Westergren techniques were routinely assessed in the laboratory. C-reactive protein (CRP), liver and renal function tests, serum C3 and C4 complement, and 24-hour urine protein levels were also assessed. Antinuclear antibodies (ANA) and anti-dsDNA antibodies were screened in SLE individuals. If indicated, renal biopsies were performed and classified [14].

Evaluating Interleukin-36α (IL-36α) in Patients and Normals:

Method of detection for interleukin36 α (IL-36 α) levels by ELISA technique [15].

Before use, all reagents and samples were brought to room temperature. It is suggested that all standards, samples, and controls be measured twice.

Prior to usage, both patient serum and control serum were diluted by a factor of 100. Do not dilute the standards.

- Sufficient micro-plate modules for all calibrators/controls, and patient samples were manufactured.
- Wells were pipetted with 100L of calibrators, controls, and pre-diluted patient samples.
- Samples were incubated at ambient temperature for 30 minutes (20-28).
- The microwell contents were discarded and cleaned three times with 300l of wash solution.

In each well, 100L of enzyme conjugate was dispensed.

- The microwell contents were discarded and cleaned three times with 300l of wash solution.
- Each well-received 100l of TMB (tetramethylbenzidine) substrate solution.
- The TMB substrate solution was incubated

for 15 minutes.

- 100 microliters stop solutions were applied to each module.
- The optical density at 450 nm was measured, and the findings were computed.
- The colour created is stable for at least thirty minutes.
- The outcome was read at this time.

Statistical analysis: For statistical evaluation. Statistical Software for the Social Sciences (SPSS) Edition 20.0 was used. Variables were shown as mean, standard deviation, or as number and percentage. The Student's t-test or Mann-Whitney U test, as applicable, was employed to gauge data. The Pearson correlation test and linear regression analysis were used. The significance value was p 0.05.

3. Results:

This was a case-control study involving 80 individuals, divided into forty cases with SLE disease and forty healthy gender- and age-balanced controls were included. 38 females (95.0%) and 2 males (5.0%) were ages 20–45, with a mean of 32.8±7.8. Controls were 19–43 years old, with a mean of 31±5.3 years. SLE patients exhibited significantly higher IL-36 levels (90.7±22.9 pg/ml) than healthy controls (27.3±9.8 pg/ml) (P < 0.001) **Table (1).**

Table 1: Demographic data of the studied groups and comparison between cases and controls regarding interleukin 36 alpha (IL36α) level:

Items	Cases (no=40)	Controls (no=40)	P-value
Age Range (min-max) (mean ± SD)	 20-45 32.8±7.8	 19-43 31±5.3	0.062
Sex Male Female	 2(5.0%) 38(95.0%)	 7(17.5%) 33(82.5%)	0.077
IL36α Range (min-max) (mean±SD)	 (40.7-124.8) 90.7±22.9	 (12.7-57.8) 27.3±9.8	<0.001*

IL36α, Interleukin 36 alpha.

Among our forty patients, the mean illness duration was 6.3±3 years; thirty-three patients (82.5%) had a malar rash, and 7 patients (17.5%) had a discoid rash. Hematologic disorders in the form of

leucopenia and/or thrombocytopenia were found in 20 patients (50.0%). Serositis in the form of pleurisy, pericarditis, and/or pericardial effusion was found in 7 patients (17.5%). Fever was found in 22 patients (55%), neurologic disorders were found in 4 patients (10%) in the form of seizures. Thirteen (32.5%) cases had renal biopsy reports. 20 patients (50%) proved positive for anti-dsDNA, whereas 40 (100%) had positive ANA **Table (2).**

Table 2: Clinical features among the studied patients:

Items	Value	
	No	**%**
Disease Duration (mean±SD) (year)	6.3 ± 3	
Malar rash	33	82.5%
Discoid rash	7	17.5%
Photosensitivity	38	95.0%
Oral ulcers	22	55.0%
Eye symptoms	3	7.5%
Arthritis	25	62.5%
Renal disorder	13	32.5%
Haematologic	20	50.0%
Serositis	7	17.5%
Alopecia	27	67.5%
Myositis	1	2.5%
Amenorrhea	0	0.0%
Abortion	1	2.5%
Associated co-morbidities		
DM	3	7.5%
HTN	4	10%
Fever	22	55.0%
ANA	40	100.0%
Anti DNA	20	50.0%
Anti PLA	3	7.5%
Neurologic	4	10.0%
Vasculitis	4	10.0%

ANA, Antinuclear antibody; Anti DNA, Anti double-stranded DNA; Anti PLA, Anti phospholipid antibody.

(37.5%) had high 24-hour urine protein, urinary casts (granular) were present in only 4 cases (10%) and both pus cells and RBCs were due to nephritis. Our patients showed SLEDAI scores from 3 to 25 with a mean of 13.3±6.1 25 cases (62.5%) had no damage, whereas 15 had damage (37.5%), as reported by the SLICC score **Table (3).**

Table 3: Laboratory characteristics, Immune profile and Activity chronicity scores of the studied patients:

NO{40}	Range (min _ max)	Mean	Std Deviation
ESR	(20.00 - 120 .00)	55.2250	24.01013
CRP	(1.00 - 14.00)	6.2000	3.04833
HG	(7.00 - 107)	16.0125	19.85605
TLC	(2.00 - 14.00)	5.7500	2.88008
PLT	(80 - 470)	236.4500	77.58269
ALT	(6.00 - 88.00)	21.2250	12.48689
CREATE	(1.00 - 2 .00)	1.1000	0.30382
UREA	(16.00 - 178.00)	44.2500	43.11270
TAG	(41.00 - 301.00)	136.9750	66.03670
Cholesterol	(29.00 - 350.00)	182.1500	70.66009
LDL	(29 - 201)	108.8500	45.24665
HDL	(15.00 - 64.00)	32.7750	8.47163
C3	(14.0 - 130.0)	59.9000	22.97245
C4	(6.00 - 46.00)	20.4250	8.85463
Urine pus cells	(0 – 45)	17.5641	13.15254
Urine RBCs	(0 - 20)	3.1250	5.36459
Protein	15 37.5%		
Casts	4 10.0%		
24h urinary Protein	(0 - 2.50)	0.4282	0.70783
SLEDAI	(3.00 - 25.00)	13.2750	6.05525
SLICR	(0.00 - 2.00)	0.4250	0.59431
0	25 62.5%		
1	13 32.5%		
2	2 5.0%		

Hb; hemoglobin TLC; Total leucocytic count ESR; Erythrocyte sedimentation rate PLT; Platelet count ALT; alanine transaminase CRP; C Reactive protein AST; aspartate aminotransferase. C3, complement 3; C4, complement 4; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICR, The Systemic Lupus International Collaborative Clinics/ACR.

IL 36α has a significant link to oral ulcers, arthritis, renal disorders, fever (P <0.05) and anti-dsDNA titre (P <0.001) **Table (4)**.

Table 4: Relation between clinical characteristics of the disease and Interleukin 36*α*:

Items	Negative	Positive	P-value
Sex **Male** **Female**	 105.4±4.7 89.9±23.2		 0.359
Malar rash	80.8±26	92.8±22.1	0.214
Discoid rash	89.7±24.1	95.1±16.6	0.578
Photosensitivity	91±15.5	90.6±23.4	0.983
Oral ulcers	79.8±23.6	99.6±18.4	0.005*
Eye symptoms	90.6±22.6	92.1±31.5	0.914
Arthritis	71.4±22.8	102.2±13.4	<0.001*
Renal disorder	83.2±23	106.2±13	0.002*
Hematologic	90.8±24	90.6±22.4	0.981
Serositis	93.1±22.4	79.3±23.3	0.152
Alopecia	81.5±26.4	95.1±20	0.168 (MW)
Myositis	90.3±23.1	104±.	0.562(MW)
Abortion	91±22.8	86.5±28.3	0.750
Fever	81.2±23.9	98.4±19.3	0.016*
Neurologic	89.1±23.5	104.8±9.1	0.199
Vasculitis	89.3±22.4	103.4±26.4	0.247
Anti dsDNA	74.9±20.9	106.3±10.9	<0.001*

*P-value is significant<0.05

IL36α had a substantial linear positive connection with age, duration of illness, ESR, CRP, urine pus cells, SLEDAI, and SLICR scores. C3 correlated negatively with IL36α **Table (5)**.

Table 5: Correlation between the IL36α and age, disease duration and laboratory criteria:

Items		Interleukin 36 alpha
Age	Pearson Correlation (r)	0.472**
	P-value	0.002
Disease Duration	Pearson Correlation (r)	0.626**
	P-value	0.000
ESR	Pearson Correlation (r)	0.680**
	P-value	0.000
CRP	Pearson Correlation (r)	0.668**
	P-value	<0.001
HG	Pearson Correlation (r)	0.140
	P-value	0.388
TLC	Pearson Correlation (r)	0.139
	P-value	0.391
PLT	Pearson Correlation (r)	0.101
	P-value	0.537
ALT	Pearson Correlation (r)	0.115
	P-value	0.479
CREATE	Pearson Correlation (r)	0.240
	P-value	0.136
UREA	Pearson Correlation (r)	0.249
	P-value	0.122
TAG	Pearson Correlation (r)	0.121
	P-value	0.456
Cholesterol	Pearson Correlation (r)	0.092
	P-value	0.571
LDL	Pearson Correlation (r)	-0.134
	P-value	0.410
HDL	Pearson Correlation (r)	-0.015
	P-value	0.927
pus cells	Pearson Correlation (r)	0.659**
	P-value	<0.001*
RBCS	Pearson Correlation (r)	0.278
	P-value	0.082

24h urinary protein	Pearson Correlation (r)	0.285
	P-value	0.079
C3	Pearson Correlation (r)	-0.710**
	P-value	<0.001
C4	Pearson Correlation (r)	-0.257
	P-value	0.109
SLEDAI	Pearson Correlation (r)	0.880**
	P-value	<0.001
SLICR	Pearson Correlation (r)	0.571**
	P-value	<0.001

*P-value is significant<0.05

IL36 alpha can predict SLE at a cut-off >50.5 with a sensitivity 95%, specificity 97.5% **Table (6)**.

Table 6: Validity data of Interleukin 36α in SLE:

Items	**Values**
Cut off	>50.5
AUC	0.993
P-value	<0.001*
Sensitivity (95% CI)	95.0(83.1 - 99.4)
Specificity (95% CI)	97.50(86.8 - 99.9)
PPV (95% CI)	97.4(84.6 - 99.6)
NPV (95% CI)	95.1(83.5 - 98.7)

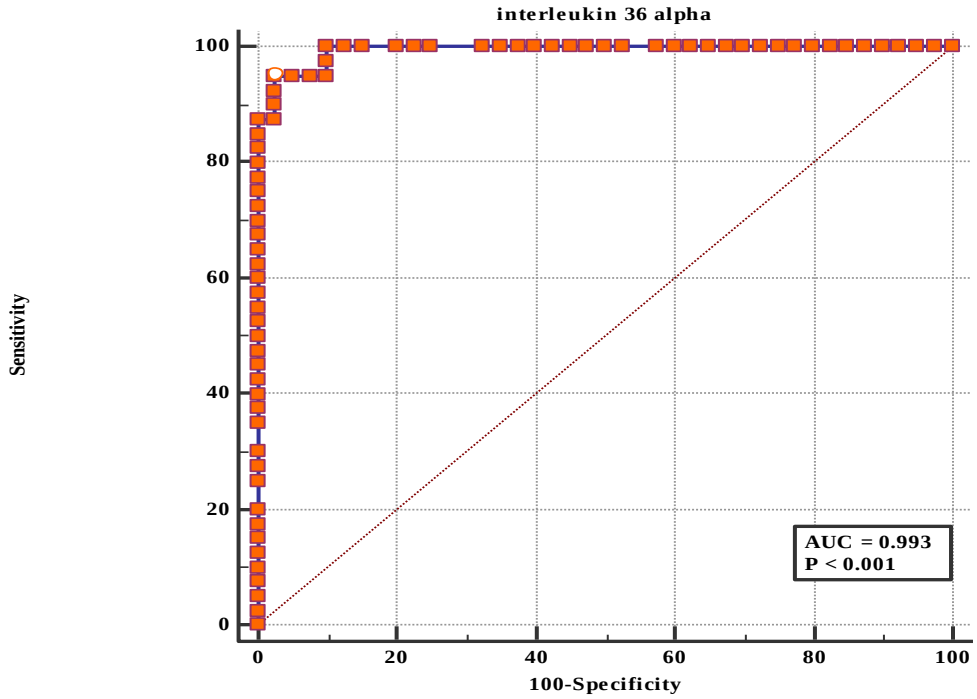

Figure (1): ROC curve for prediction of SLE

4. Discussion:

SLE is especially prevalent in women aged 20 to 40, particularly those of African and Latino descent. It is related to a tripled mortality risk [16]. The pathophysiology of SLE is complicated and ambiguous, and novel therapeutic options are gradually developing [17].

IL-36 signaling stimulates immune cells. DCs connect the innate and adaptive immune systems by balancing immunological tolerance and autoimmune inflammation [18]. DCs prime helper T (Th) cell differentiation [19]. IL-36 agonist over-expression or down-regulation of IL-36Ra is linked to inflammatory alterations in the afflicted tissues [20]. It is strongly hypothesized that IL-36 has an impact on SLE pathogenesis [21].

The purpose of our research was to analyze the Interleukin 36 levels in SLE individuals and their link with SLE activity. In this research, there were 38 female and 2 male patients. Regarding the clinical data of SLE patients, 33 patients (82.5%) had malar rash, 7 patients (17.5%) had discoid rash, 38 patients (95.0%) had photosensitivity, 22 patients (55.0%) had oral ulcers, 27 patients (67.5%) had alopecia, and 25 patients (62.5%) had arthritis. Thirteen (32.5%) cases had renal biopsy reports.

Wang et al. 2021[22] identified the following clinical characteristics in 60 Chinese SLE patients: 60% exhibited malar rash, 36.6% arthritis, 21.6% oral ulcers, 75% proteinuria, 28.3% lymphopenic, 40% thrombocytopenic, 10% pericarditis, 16% pleuritis, and 6.6%

neurological symptoms. Comparing our data to others shows that organ involvement in SLE patients varies. Sample size, demographics, clinical factors, and therapy may have caused these differences.

Our case-control study found a significant difference in blood IL-36 levels between our patients and controls. Patients had 90.7±22.9 pg/ml IL-36 and healthy controls 27.3±9.3 pg/ml (P< 0.001). **Wang et al. 2021** used ELISA to measure 36 in healthy people and SLE patients, supporting our results. SLE patients had greater serum IL36 levels than healthy controls 50 (39 - 108) pg/m vs. 26 (17 - 32) (P<0.001).

Wong and colleagues 2015 [23] detected higher IL-36 levels in 43 SLE individuals, contrary to 60 controls (p< 0.05). In a separate study, **Elsiss and colleagues** [24] compared 84 patients to 84 healthy participants, confirming our findings. SLE patients reported considerably higher serum IL-36α compared to controls. SLE patients had 65.6 ± 39.1 pg/ml of serum IL-36α, compared to 37.9 ± 17.2 in controls (p < 0.001).

Zhang and colleagues [25] showed that SLE patients had similar blood IL-36 levels to controls, contrary to our findings (P > 0.05)**.** Inconsistent findings may have various causes. First, our study patients were the most active. Autoimmune disorders also include IL-36α, not all IL-36 cytokines.

We analyzed the link between SLE patients' blood IL-36α levels and disease activity utilizing the SLEDAI rating system. They correlated favorably (r=0.880, P< 0.001).

Supporting our research, **Mai and colleagues 2018** [26] found a significantly positive link between SLE illness activity (by SLEDAI rating) and IL-36α levels (r = 0.308, P = 0.008).

In SLE patients, **Wang and colleagues 2021** observed a favourable link between SLEDAI rating and IL-36α levels (r= 0.374, P= 0.003). **Wong et al. 2015** found a favourable association between SLEDAI rating and IL-36α levels in 43 SLE patients (r = 0.382 a, p < 0.05).

Mohamed et al. 2021 [27] showed that IL-36α levels were increased with SLE activity and substantially linked to SLEDAI. In Chinese SLE patients, SLEDAI and IL-36α were unrelated (**Zhang et al. 2021).** Disparities may be caused by sample size and disease control.

In our investigation, oral ulcers, fever, and arthritis had substantially greater IL-36α mean blood levels than those without (P-value =0.005, 0.016, and <0.001).

Wang et al. 2021 discovered that individuals with mucocutaneous involvement had greater blood IL-36α levels (p = 0.003). **Mai and colleagues 2018** discovered increased serum IL-36α with active arthritis in SLE patients (P < 0.001). **Elsiss and colleagues 2022** spotted that SLE patients with active arthritis detected by musculoskeletal ultrasound (MSUS) (synovitis and/or erosion) had higher blood

IL-36α levels (p<0.001). **Mohamed et al. 2021** discovered that individuals with mucocutaneous involvement had higher IL-36α levels (4.1±1.4 vs 2.8±2.1, P=0.041).

Unlike our investigation, **Elsiss et al. 2022** observed no link between constitutional, mucocutaneous, neuropsychiatric, or cardiac symptoms and IL-36α levels (P>0.05).

Our lupus nephritis (LN) patients had IL-36α levels of 106.2±13 (p=0.002). **Wang et al. 2021** reported increased IL-36 levels in lupus nephritis individuals (r= 0.329, P= 0.010), supporting our findings. IL-36α levels didn't correlate with nephritis, unlike our investigation (P > 0.05) **(Mai et al. 2018).**

In our cases, ESR and CRP were linked to IL-36 levels (P =0.001). **Wong et al. 2015** showed that higher ESR was linked with high serum IL-36α (P =0.001). **Wang and colleagues 2021** and **Elsiss et al. 2022** observed no apparent link between ESR or CRP and IL-36α levels in 84 SLE patients (P= 0.14, 0.16).

Anti-dsDNA antibody-raised titre individuals exhibited greater mean IL-36α (p <0.001). In **Wang et al. 2021**, individuals with increased anti-dsDNA antibodies had higher IL-36α blood levels (p = 0.019). IL-36α and anti-dsDNA were unrelated in **Elsiss et al. 2022.**

We identified a substantial linear negative association between IL-36α serum concentrations and C3 serum levels in SLE patients (p <0.001). C4 concentration and serum IL-36α concentrations did not correlate in SLE patients (p = 0.109). **Mai et al. 2018** found that C3 and IL-36α were negatively linked in lupus patients (p= 0.019).

Wang et al. 2021 discovered a negative link between C3 and IL-36α (P =0.009). **Wong et al. 2015** and **Elsiss 2022** found conflicting findings. Both investigations demonstrated no link between C3 or C4 levels and IL-36α levels in SLE patients.

Our study showed no noteworthy disparities in mean serum IL-36α levels between patients with proteinuria, hematuria, or urinary casts over those without (p =.079 and .082 respectively), but there was a positive correlation between pus cells in urine and serum levels (p<0.001). **Elsiss et al. 2022** found no association between serum ILa-36α levels and proteinuria or urinary casts, supporting the current investigation. However, **Wang et al. 2021** related proteinuria and hematuria to IL-36α levels (p= 0.037 and 0.27, respectively).

In our investigation, SLE patients' age and illness duration were linked to IL-36α levels (p =0.002 and 0.001). Conflicting our findings, **Elsiss et al. 2022** found that illness duration and IL-36α levels were unrelated (p=0.4 and 0.3).

Conclusion and Recommendations:

- We suggest interleukin 36 alpha for SLE diagnosis and prognosis.

• Interleukin 36α may identify SLE disease activity early during follow-up.

• Active lupus nephritis raises serum interleukin 36α, which may be utilized for follow-up.

• SLE therapy with IL 36α antagonism needs further investigation.

• Further trials must be conducted to verify such findings

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