



# Interleukin 25 and Systemic Lupus Erythematosus

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### Abstract

**Objectives:** We identified that type 2 immunity stimulation has been associated with the pathogenesis of Systemic lupus Erythematosus (SLE), so we studied serum level of IL 25 and its relation to SLE activity, clinical and laboratory characteristics.

**Methods:** Serum from 90 patients who met the American College of Rheumatology (ACR) criteria for SLE and 40 healthy controls was tested for IL 25 level by Enzyme Linked Immunosorbent Assay (ELISA). Clinical and laboratory characteristics and disease activity using SLE Disease Activity Index (SLEDAI) were also assessed.

**Results:** IL 25 serum level was significantly higher among SLE patients than healthy controls. It has statistically significant correlation with disease activity.

**Conclusion:** Our data indicates that IL 25 level is elevated in SLE patients in comparison to healthy controls and is correlated to high disease activity, potentially being a biomarker predictive for disease activity in SLE.

### Keywords

Interleukin 25; Systemic lupus erythematosus; Lupus nephritis; Systemic lupus erythematosus disease activity index.

## Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease happen with diverse autoantibodies against self-antigens and affects different organ systems. It has been identified that dysfunctions of the immune system, such as imbalance between Th1/Th2 cytokines is an important factor in SLE pathogenesis [1]. There are contradictory data about predominance of responses by Th1 or Th2, but a rise in Th2 responses is apparent in SLE [2].

Interleukin-17 (IL 17) is a member of a new cytokine family that has recently gained prominence due to its involvement in autoimmune diseases in both human and mouse. There are six members in IL-17 family involving IL 17A, IL 17B, IL 17C, IL 17D, IL 17E (known as IL 25), and IL 17F [3]. While IL 25 has a unique function and structure [4].

Nearly all IL-17 family members have pro-inflammatory role, whereas IL 25 has a different function which stimulate the development of type 2 immune responses (Th2) [5]. IL 25 has receptors (IL-17BR) which are greatly expressed in vivo by stimulation of Th2 cells [6].

Fort et al., [2001] [7] was the first one who determines IL 25 by a study performed to analyze the sequence homology in cytokine IL-17 DNA. The sequence of IL 25 is 16-20% consonant to that of IL-17, exposing its particular function. Actually, this cytokine has a stimulatory effect for Th2 cells [8]. IL 25 can also have stimulated the secretion of other cytokines. For example, production of IL 25 in human and mice or injection and induction of IL 25 to animals caused the secretion of high levels of Th2 cytokines, involving IL 13, IL 4, and IL 5 [9]. In addition, by stimulation of transcription factor NF-KB, IL 25 activates the production of IL 8 cytokine in kidney cells. However, the function of this cytokine in renal diseases is still not being interpreted [10]. This cytokine also increases serum secretion of IgA, IgG1 and IgE antibodies, blood eosinophil's, and eosinophil aggregation in liver [11].

As most autoimmune diseases arise from disruption of balance between the immune cells, in case the autoimmune disease arises from Th1 cells, injection of IL-25 advances differentiation of Th0 to Th2 cells and assembly of their cytokines can be effective in amelioration of the disease [12].

In this study, we investigated the expressions of IL 25 in the serum of SLE patients and evaluated the correlation between serum IL 25 levels and clinical and laboratory values, as well as severity of SLE patients.

## Subjects and Methods

### Subjects

Ninety SLE cases and 50 healthy controls were included in this work. All cases were admitted to Rheumatology and Rehabilitation Department, Assiut University, Egypt. All subjects met the 1982 and 1997 American College of Rheumatology (ACR) criteria for SLE [13] at the time of recruitment, and were divided into two groups: 40 patients with LN and 50 patients with SLE according to the laboratory data. Controls were sex and age matched, and had no apparent history of SLE, all controls had normal ESR, meaning that they represent a non-inflamed subject. The patients' written consent was obtained according to the declaration of Helsinki and the study has been approved by local ethics committee prior to their inclusion in the study.

### SLE clinical and laboratory characteristics

SLE disease activity was measured by the same physician (SM) in all patients, using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [14]. Active LN was described as active urinary sediment and cellular casts, and/or worsened glomerular filtration rate (GFR) >25 % above baseline/normal range caused by active LN and/or proteinuria  $\geq 0.5$  g/day, and/or C3 hypocomplementemia. At least two of the previous criteria had to be found and renal biopsy showing lupus nephritis. Sixty five patients (72.2%) had arthritis, 40 (44.4%) patients had a diagnosis of SLE-renal disease, 4 (4.4%) patients had Seizures, 5 (5.5%) patients had psychosis 31(34.4%) patients had Malar rash, 16(17.7%) patients had oral ulcers, 9(10%) patients had alopecia (Table 1). Additional measurements included complete blood picture(Beckman Coulter, California, United States), urine analysis (Sysmex UF-1000<sup>TM</sup>

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**Table 1:** Clinical and laboratory characteristics of SLE patients.

Characteristics	SLE n=(90 )
Disease duration, years, mean ± SD	6.13 ± 1.25
<b>SLEDAI categories, n (%)</b>	<b>15(16.7%)</b>
Mild	34(37.7%)
Moderate	41(45.6%)
Severe	
Anti-dsDNA Ab positive, n (%)	42(46.6%)
C3 consumed, n (%)	39(43.3%)
C4 consumed, n (%)	36(40%)
CRP mean ± SD	5.3 ± 1.3
Proteinuria ≥ 0.5 mg/24 h, mean ± SD	205 ± 99
Hematuria, n (%)	11(12.2%)
Urinary casts, n (%)	18(20%)
Arthritis, n (%)	65(72.2%)
Malar Rash, n (%)	31(34.4%)
Oral ulcer, n (%)	16(17.7%)
Seizures, n (%)	4(4.4%)
Psychosis, n (%)	31(34.4%)
Alopecia, n (%)	9(10%)
Leucopenia, n (%)	8(8.8%)
Thrombocytopenia, n (%)	18(20%)
Anemia, n (%)	42(46.6%)
Lupus nephritis, n (%)	40(44.4%)

Automated Urine Particle Analyzer), (urinary 24 hour protein, urea, creatinine, creatinine clearance, glomerular filtration rate, liver function test, lipogram){ using chemiluminescence technology (Dimension EXL 200)}, antinuclear antibodies were quantified using indirect immunofluorescence assay (IFA, ANA-HEp2-IgG, VIRO-IMMUN Labor Diagnostika, GmbH, Oberursel, Germany) and were considered significant when titers ≥ 1:80; anti-dsDNA antibodies were quantified using indirect immunofluorescence assay (NOVA Lite<sup>®</sup> dsDNA Crithidia luciliae, Inova Diagnostics, San Diego, California) and were considered significant when titers ≥ 20 IU/, serum complement 3(C3), complement 4(C4) using nephelometric assay (Behring Nephelometer II, Dade Behring, Marburg, Germany) and high sensitivity C-Reactive protein(hsCRP) using nephelometric assay (Behring Nephelometer II, Dade Behring, Marburg, Germany).

### Serum cytokine quantification

Serum samples were withdrawn from each patient and control stored at -20°C until tested. Serum IL-25 levels were analyzed by ELISA kits according to the manufacturer’s protocols (Human IL-25 ELISA reagent kit [Elab Science], Hon- gshan district, wuhai province, china). The results were represented as picograms per milliliter (pg/ mL). All samples were tested in duplicates.

### Statistics

The data were tested for normality using the Anderson-Darling test and for homogeneity variances prior to further statistical analysis. Categorical variables were described by number and percent (N, %), where continuous variables described by mean and standard deviation (Mean, SD). Chi-square test and fisher exact test used to compare between categorical variables where compare between continuous variables by t-test and Independent-Samples T test, Mann-whitney U, kruskal wallis test. A two-tailed p < 0.05 was considered statistically

significant. All analyses were performed with the IBM SPSS 20.0 software.

### Results

The age of 90 subjects with SLE included in the study ranged from 22.5 to 43 years with a median of 28years. All of them are females (100%) for SLE patients. No statistically significant difference was observed between SLE patients and controls concerning age and sex.

We tested IL 25 in 90 SLE patients and 50 controls. Serum levels of IL 25 were significantly increased in the SLE group (268.24 ± 138.03 pg/mL) compared to the control group (43.83 ± 15.42 pg/mL, P=0.001) (Figure 1).

In our study, we have detected significant positive correlation between high serum IL 25 and Anti ds DNA and C3 (p=0.025, r=0.456) and (p=0.021, r=0.388) respectively, while there is negative correlation between high serum IL 25 and CRP (p=0.027, r=-0.392) (Table 2). Furthermore, linear regression stated that high IL 25 is associated with Anti ds DNA also in an additive model, which remained strongly significant after adjustment for accompanying characteristics, the results given as odds ratio {95% Confidence interval}: 2.9{ 1.88-5.4} P=0.002 (Table4).

However, no significant associations were observed between high serum IL 25 and either the following laboratory and clinical characteristics: ANA, ESR, complete blood picture, Lipogram,C4, creatinine, oral ulcers, arthritis, vasculitis, pericarditis, pleurisy and psychosis (Table 2).

A significant correlation was observed for elevated serum IL 25 with clinical disease activity scores (P = 0.011, r = 0.55) as well as different SLEDAI categories (Table 3 and Figure 2).

Further analysis was made for the data of SLE patients to investigate the association of serum IL 25 with renal involvement. SLE patients were divided into two groups according to the presence of renal involvement, but we did not find a significant correlation between serum IL 25 and lupus nephritis.

### Discussion

Currently, many studies reported the role of IL 25 in autoimmune diseases as an inhibitor of both adaptive and innate immunity and an anti-inflammatory cytokine [15]. In organ-specific autoimmunity, the equivalence of cytokines is an essential determinant of susceptibility and resistance. There is an important argument in SLE which is the imbalance between Th1/Th2 cytokines. However, there are conflicting opinions about superiority of responses by Th1 or Th2, a rise in Th2 responses is noticeable in SLE [16]. In SLE, the secretion of prion inflammatory cytokines, for instance IFN γ, TNF α, IL 6 and IL 17 is observant to associate with disease susceptibility. Nevertheless, Th2 cytokines, like IL 13, IL 4 and IL 5, have been revealed to take an important role in alleviating and even prohibiting autoimmune diseases [17].

In the present study, all SLE cases are females which can be explained by the increased susceptibility may be due to differences in hormones and sex chromosomes [18]. We studied serum IL 25 level, a Th2 stimulating cytokine in SLE patients and analyzed their correlations with laboratory and clinical characteristics. Our results found significant high level of IL 25 serum in SLE patients when compared to controls (Table 2).

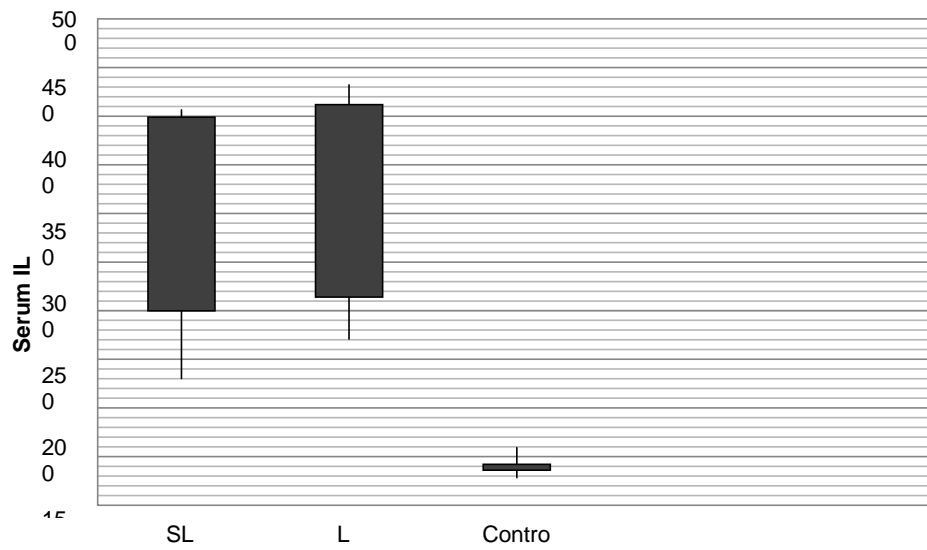


Figure 1: Comparison of interleukin 25 (IL 25) level in systemic lupus erythematosus (SLE) patients, Lupus Nephritis (LN) and healthy controls.

Table 2: Comparison of interleukin 25 (IL 25) level in systemic lupus erythematosus (SLE) patients, Lupus Nephritis (LN) and healthy controls.

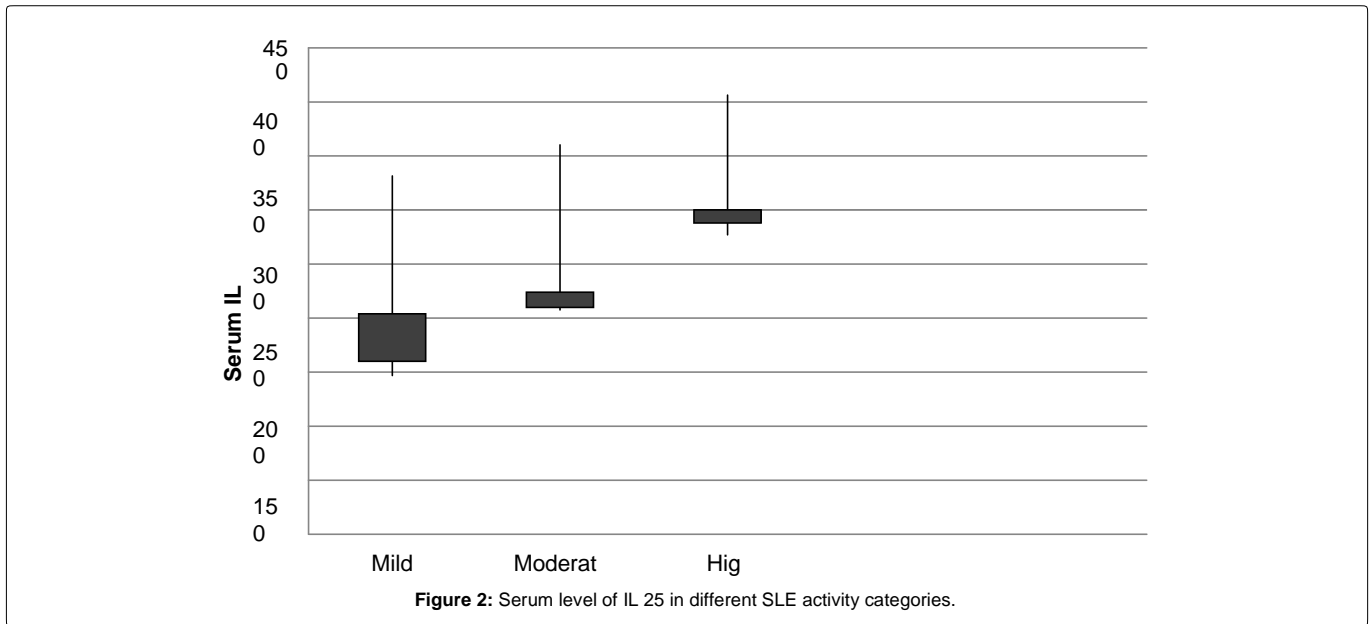
	SLE	LN	Controls	P value
Serum IL25(pg/mL)	268.24 ± 138.03	291.3 ± 120.5	43.83 ± 15.42	0.001**

Note: Statistically non-significant correlation (p > 0.05); \*Statistically significant correlation (p < 0.001)

Table 3: Laboratory characteristics of SLE patients with regard to the serum IL 25 level.

Characteristic	High >200pg/ml	Low <200pg/ml	P.value
	Median(Range)	Median(Range)	
Age(years)	32.5(27.25 - 45)	34(32 - 42)	0.257
Duration (years)	6(4 - 6)	3.1(1.5 - 11.5)	0.549
ESR(1 <sup>st</sup> hr)(mm/hr)	64(21.5 - 90)	50.5(30.75 - 67.5)	0.419
ESR (2 <sup>nd</sup> hr)(mm/hr)	83(42.5 - 115)	71(49.75 - 98.5)	0.234
CRP(mg/L)	2.5(1.55 - 11.35)	4.5(1.35 - 13.3)	<b>0.027*</b>
ANA(AI)	34(31.75 - 52.45)	14.4(3.78 - 42.77)	0.285
Anti-ds DNA(IU/ml)	123(17.3 - 200)	63.5(18.9 - 167.5)	<b>0.025*</b>
Hb(g/dL)	10.5(9.35 - 12.05)	11.5(8.55 - 12.53)	0.828
WBCs(10 <sup>3</sup> /µl)	5.78(2.95 - 9.5)	5.47(5 - 7.16)	0.878
PLTs (10 <sup>3</sup> /µl)	205.4(166.4 - 259)	231.5(167.25 - 329)	0.721
AST(U/L)	25(19 - 29.5)	25(14 - 35.5)	0.852
ALT(U/L)	18(14 - 29)	17(7 - 31)	0.689
S. Cholesterol(mg/dl)	181.5(122.5 - 197.75)	202(149.5 - 223)	0.444
Triglycerides(mg/dl)	111(63 - 172.75)	141(124 - 208)	0.285
HDL(mg/dl)	57.5(50.5 - 69.75)	48(37 - 51)	0.141
LDL(mg/dl)	121.15(72.75 - 133)	127(99.3 - 148.65)	0.543
C3(g/L)	0.55(0.35 - 1.12)	0.81(0.82 - 0.97)	<b>0.021*</b>
C4(g/L)	0.14(0.08 - 0.2)	0.26(0.26 - 0.7)	0.052
Urine WBCs(hpf)	10(0 - 28.75)	0(0 - 32.5)	0.633
Urine RBCs(hpf)	1(0 - 3.75)	5(0 - 31)	0.438
Urine 24hr Protein(mg/24hr)	191.5(28.5 - 730.5)	200(98.8 - 1655.5)	0.960
Creatinine Clearance(ml/min)	98.15(67.5 - 129.5)	98.5(62 - 143.3)	0.496
S. creatinine(µm/L)	64.5(33.13 - 88.5)	58(47.5 - 74.52)	0.894
eGFR (ml/min/1.73m <sup>2</sup> )	99.5(65.25 - 118.25)	108(81.5 - 141.5)	0.515

Note: Statistically non-significant correlation (p > 0.05); \*Statistically significant correlation (p < 0.05)



**Table 4:** Interleukin 25 serum levels in different Systemic Lupus Erythematosus Disease Activity Index categories.

Disease activity score	Mean (SD) pg/mL	P-value
Mild	239.12 ± 92.33	0.031*
Moderate	283.81 ± 76.34	
High	341.57 ± 64.63	

**Note:** Significant correlation (p=0.031) was found between level of serum IL 25 and SLE activity score (Mild, Moderate and High scores)

**Table 5:** The effect of high IL 25 on the risk of SLE analyzed by logistic regression.

Serum IL 25	Crude OR (95% CI) p- value	Adjusted OR (95% CI) p- value
High serum IL 25	2.9(1.88-5.4) 0.012	2.1(1.45-4.5) 0.015
Low serum IL 25	0.84(0.35-1.9) 0.456	0.76(0.25-1.5) 0.332

**Note:** OR denotes odds ratio; CI, confidence interval; Adjusted for laboratory characteristics of SLE (ANA, CRP, C3 and C4).

To study the associations between disease biomarkers and serum IL 25 levels, we studied correlations between laboratory markers and IL 25, including anti-dsDNA, ANA, CRP, consumed C3 and C4. We found significant positive correlation between serum IL 25 and ds DNA and consumed C3. While there is significant negative correlations with CRP levels. On the other hand, we did not find significant correlation between serum IL 25 and the other markers (Table 3).

Stated that IL 25 level was increased in both of serum and synovial fluid of Rheumatoid Arthritis (RA) patients when compared to osteoarthritis (OA) patients and controls [19]. In this study we obviously found that IL 25 levels were significantly higher in SLE patients with active disease than in patients with inactive disease. Also, revealed that higher serum and synovial fluid levels of IL 25 were found in RA patients with severe and moderate disease activity when compared to those patients with low disease activity and with obvious comparison to patients in remission [20].

Previous study reported that IL 25 has an important role on treatment of autoimmune diseases in kidney patients [21], for example Adriamycin nephropathy (AN) which is a kidney disease characterized by proteinuria. This disease is aggravated by Adriamycin. Qicao et al., 2011 found that treatment of the cases with IL 25, the urinary loss of proteins significantly lower than in the mice without treatment [22].

So, other studies are needed to be done on SLE to further explain the role of IL 25 in the pathogenesis and treatment of these diseases.

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