Original article

Serum interleukin 27: a possible biomarker of pediatric systemic lupus erythematosus

Background: *Systemic lupus erythematosus (SLE) is a complex autoimmune disease; different cytokines play a role in the immunopathogenesis of SLE. IL-27 has both immunosuppressive and pro-inflammatory roles and its role is unclear in SLE.*
Objectives: *To measure serum interleukin (IL)-27 among a group of patients with pediatric SLE (pSLE) and whether it varies with SLE clinical and laboratory features or with therapy.*
Methods: *Fifty patients with pSLE and 25 healthy subjects were included. Routine laboratory and immunological markers of SLE were done. Serum IL-27 was measured by enzyme linked immunosorbent assay for both patients and healthy subjects.*
Results: *Serum IL-27 was significantly lower in patients when compared to healthy subjects ($p< 0.001$); 17 patients (34%) had low serum IL-27 (serum IL-27 < 160 pg/ml). Patients in lupus flare and those in remission had comparable levels ($p> 0.05$). Serum IL-27 did not vary significantly between patients with lupus nephritis (LN) and those without evident LN, moreover, it was comparable among different histological classes of LN ($p> 0.05$). The disease status in terms of SLE disease activity index was comparable among lupus patients with normal serum IL-27 and those with decreased serum IL-27 ($p> 0.05$). Serum IL-27 was not affected significantly with the cumulative doses and the types of the immunosuppressive drugs used ($p> 0.05$).*
Conclusion: *Decreased serum IL-27 in SLE might support its involvement in the immune alteration underlying SLE but its exact role remains unclear.*

Key words: Interleukin-27, Lupus nephritis, Pediatric lupus.

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INTRODUCTION

Interleukin (IL)-27 is a new member of the IL-12 family of heterodimeric cytokines composed of the Epstein Barr virus-induced gene 3 (EBI-3) and p28 subunits[1]. IL-27 was shown to regulate various molecules associated with the function and maintenance of T-helper 17 (Th17 cells)[2]. Th17 cells are found to be implicated in the pathogenesis of systemic lupus erythematosus (SLE)[3].

SLE is a complex auto-immune disorder which involves various facets of the immune system. In addition to autoantibody production and immune complex deposition, emerging evidences suggest that cytokines may act as key players in the immunopathogenesis of SLE. These cytokines assume a critical role in the differentiation, maturation and activation of cells and also participate in the local inflammatory processes that mediate tissue insults in SLE[4].

It has been demonstrated that IL-27 plays a key role in human T-cells by promoting IL-10-secreting Tr1 cells and inhibiting Th17 cells and thus provides a dual regulatory mechanism to control autoimmunity and tissue inflammation[2]. It has been reported that deficiency in EBI-3 in MRL/lpr mice results in pathological alteration of autoimmune glomerulonephritis (GN)[5]. In contrast, transgenic over expression of the WSX-1 gene in T-cells of MRL/lpr mice completely suppressed the development of GN and improved the survival rate of the mice.

However, whether IL-27 plays a role in the pathogenesis of SLE and which property it works with are still unclear[6]. So, we aimed in this study to evaluate serum IL-27 among a group of children and adolescents with SLE and whether it varies with different disease characteristics and the immunosuppressive drugs given.

METHODS

Study design and population:

This is a case-control study which included 50 patients with pediatric SLE (pSLE) fulfilling at least four of the revised American College of Rheumatology "ACR" classification criteria for SLE[7]. The study was approved by the Ethical Committee of the Department of Pediatrics, Ain Shams University, Cairo, Egypt.

The patients were enrolled into two groups: Active and inactive SLE based on SLE status as assessed by systemic lupus erythematosus disease activity index (SLEDAI) where no activity is indicated by SLEDAI equals zero[8].

Group I: Inactive SLE:

They were 25 patients, of these patients 22 (88%) were females and 3 (12%) were males. Their ages ranged between 4 and 18 years with a mean±SD of 13.3±3.7 years.

Group II: Active SLE:

It included 25 patients, they were 22 (88%) females and 3 (12%) males. Their ages ranged between 8 and 18 years with a mean±SD of 13.8±2.3 years.

- SLE patients were compared to a group of 40 healthy subjects without clinical manifestations or family history suggestive of autoimmune disorders. Their ages ranged between 4 and 14 years with mean ±SD of 9.3±2.7 years. They were 21 females (52.5%) and 19 males (47.5%).

Informed consents were taken from all participants and their caregivers.

Study measurements:

I- Clinical evaluation:

History taking with special emphasis laid on the age at onset, duration and therapeutic history in addition to full clinical examination were done to assess disease status and any associated morbidity. Cumulative doses of steroids were determined where all forms of corticosteroids given were converted to equivalent dose of prednisone [9]. Also, the cumulative doses of azathioprine and cyclophosphamide were calculated. Assessment of SLE activity was done using SLEDAI while SLE damage was assessed using Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI).

II- Laboratory investigations:

- Routine laboratory investigations for SLE included complete blood count using Coulter counter model Gene S (Coulter Corporation, HIELAS, Florida, and USA); Erythrocyte sedimentation rate (Westergren green method). Four mls of blood were collected in a tube to be clotted and centrifuged; serum was collected and stored at -20°C till the time of use. Serum was used for measuring serum creatinine level (on synchron CX7 autoanalyzer, Beckman Instruments, Brea, California, USA), antinuclear antibody (ANA), anti-double stranded deoxy ribonucleic acid antibody (anti-dsDNA) by indirect immunofluorescent (IMMCO Diagnostics, USA), complement-3 (C3) using MININEPH TM (The Binding Site Ltd Birmingham, UK.). Anticardiolipin IgM and IgG antibodies (Abs) were detected by enzyme linked immunosorbent assay (ELISA) (DRG instruments Gmbh). Twenty four hours urine protein level was assayed (on synchron CX7 autoanalyzer, Beckman Instruments, Brea, California, USA).

- In both patients and control groups: Human IL-27 was detected by ELISA (ID ELISA Biotechnology TM Human IL-27 P28 ELISA Kit. www.idlabs.com.idinfo@idlabs.com). A reference curve is used to determine the concentration of IL-27 in unknown specimens. The sensitivity for detection is less than 12.8 pg/ml.

Statistical analysis:

Analysis of data was done using the statistical package for social science (SPSS) version 15. Numeric data were expressed as mean ± standard deviation & median (range) when appropriate and qualitative data were expressed as frequency and percentage. Chi-square or Fisher exact test were used to compare qualitative variables. Comparison between two continuous variables was done using student t-test for normally distributed variables while its non-parametric analogue Mann Whitney test was used for not normally distributed ones. Comparison between three continuous variables was done using one way analysis of variance (ANOVA), followed by post hoc Bonferroni adjustment to detect significant pairwise comparison. Receiver operating characteristic (ROC) curve was used to determine the best cut off level for serum IL-27in healthy subjects. All tests were 2 tailed, p-values less than 0.05 were considered significant and less than 0.001 were considered as highly significant.

RESULTS

Demographic data and SLE characteristics of the studied patients is shown in table 1:

Among patients with active SLE, 17 (68%) patients had mild disease activity with SLEDAI ranging between 2 and4 with a median of 4; 5(20%) patients had moderate disease activity where SLEDAI ranged between 6 and10 with a median of 8. The remaining 3 (12%) patients had high disease activity with SLEDAI ranging between 12 and18 with a median of 12. Nineteen (76%) patients had

biopsy proven lupus nephritis (LN) (12 had LN class II, 5 had LN class III, one had LN class IV and another 1 had LN class V) while the remaining 6 (24%) patients did not show any clinical or urinary abnormality suggestive of LN.

All patients with inactive SLE had SLEDAI score 0. Twenty-two (88%) patients had biopsy proven LN (10 had LN class II, 10 had LN class III and the remaining 2 had LN class IV) while the remaining 3 (12%) patients did not show any clinical or urinary abnormality suggestive of LN.

Variation of serum IL-27 among the study groups:
The studied lupus patients whether active or inactive had significantly lower serum IL-27 when compared to the control group. Lupus activity did not affect significantly serum IL-27, where the studied patients with active lupus had comparable serum levels of IL-27 to those with inactive lupus (table 2). The cut off level of serum IL-27 in the healthy subjects was ≥ 160 pg/ml with sensitivity 98 % and specificity 66%, so, levels below 160 pg/ml were considered abnormally low.

The frequency of patients with active or inactive lupus among patients with decreased serum IL-27:
Seventeen patients (34%) had decreased serum IL-27 (serum IL-27 < 160 pg/ml). Of these patients, 8 (16%) had active SLE while the remaining 9 (18%) patients had inactive SLE.

The relation to age and gender:
There was no age difference between SLE patients with normal serum IL-27 and those with decreased serum IL-27 ($p>0.05$). The serum levels of IL-27 of the studied patients correlated negatively with their ages (figure 1). This was not the case with the control group ($r=0.2$, $p>0.05$). Serum IL-27 did not vary significantly between male and female patients ($p>0.05$).

Effect of duration of SLE and the age at its onset:
Both the duration of SLE and the age at its onset did not vary significantly between SLE patients with normal serum IL-27 and those with decreased serum IL-27 ($p>0.05$).

The relation to LN and its different histological classes:
Serum level of IL-27 was comparable among the studied patients with and without LN (table 3) and it did not vary significantly among between proliferative LN (LN class III and IV) and non-proliferative LN (LN class II) (table 4), only one patient with LN class V was included and found to have decreased serum IL-27. Also, the studied patients with decreased serum IL-27 had comparable frequency of LN as compared to those with normal serum IL-27 ($x^2= 0.68$, $p>0.05$). Similarly the frequency of different histological classes of LN was comparable between both groups ($x^2=0.78$, $p>0.05$).

Renal functions in terms of serum creatinine, creatinine clearance and 24 hours urinary protein were comparable among the studied patients with decreased serum IL-27 and those with normal serum IL-27 ($p>0.05$).

The variation of immunological markers (anti-dsDNA, C3, lupus anticoagulant and anticardiolipin Abs) and ESR with the status of serum IL-27 :
Patients with decreased serum IL-27 had comparable serum levels of the studied serum immunological markers and ESR to those with normal serum IL-27 ($p>0.05$). However, while the studied immunological markers did not correlate significantly with serum IL-27 ($p>0.05$), ESR was found to have a significant positive correlation with serum IL-27 ($r=0.29$, $p=0.03$).

SLEDAI and SDI with serum IL-27:
The disease status in terms of SLEDAI was comparable between lupus patients with normal serum IL-27 and those with decreased serum IL-27 ($z= -0.49$, $p>0.05$). However, patients with decreased serum IL-27 had significantly higher SDI as compared to those with normal serum IL-27 (table 5).

There was no significant difference in the cumulative doses of steroids, azathioprine and cyclophosphamide given to patients with normal serum IL-27 and those with decreased serum IL-27 ($p>0.05$).

Table 1. Demographic data and SLE characteristics of the studied patients.

	Inactive SLE group n(%)=25(50%)	Active SLE group n(%)=25(50%)
Age (years)		
Mean±SD	13.3 ± 3.7	13.8 ± 2.3
Range	4-18	8-18
Age at onset (years)		
Mean±SD	10.96±2.64	10.56±2.82
Range	3-14.5	3-16
Sex		
Male n(%)	3 (12.0%)	3 (12.0%)
Female n(%)	22 (88.0%)	22 (88.0%)
Duration (months)		
Median	36	24
Range	5-96	4-132
System involvement		
LN	22(88%)	19(76%)
NPSLE	1(4%)	2(8%)
2ry APS	0	2(8%)
Thrombocytopenia	1(4%)	1(4%)
Ulcerative colitis	1(4%)	0
Carditis	0	2(8%)
SLEDAI		
Median	0	4
Range	0	2-18
SDI		
Median	0	0
Range	0-1	0-2
Cumulative corticosteroid dose (grams)		
Mean±SD	14.3±10.8	15.9±17.3
Range	3-55.2	3-89
Cumulative azathioprine dose (grams)		
Mean±SD	7.5 ± 13.9	8.7 ± 16.9
Range	0-49	0-54
Cumulative cyclophosphamide dose (grams)		
Mean±SD	1.3 ± 2.0	2.4 ± 3.6
Range	0-6.5	0-10

LN: lupus nephritis, NPSLE: neuropsychiatric syndromes of systemic lupus erythematosus, 2ryAPS: secondary antiphospholipid syndrome, SLEDAI: systemic lupus erythematosus disease activity index, SDI: Systemic lupus international collaborating clinics/American college of rheumatology damage index, n: number, %: percentage.

Table 2. Variation of serum IL-27 among patients with active or inactive lupus and the control group.

Serum IL-27 (pg/ml)	Inactive lupus group n(%) = 25(50%)	Active lupus group n(%) = 25(50%)	Control group n(%) = 40(100%)	F	P
Mean±SD	152 ± 90.2	179.2±126.6	347.5±108.7	31.125	0.000
Range	10.00-300.00	10.00-500.00	160.00-550.00		
Post Hoc Tests	**Control vs Inactive group** P = 0.000	**Control vs active group** P = 0.000	**Inactive vs active group** P = 1.000		

SLE: Systemic lupus erythematosis, IL: Interleukin, n: number, %: percentage, SD: standard deviation, P <0.01: highly significant.

Table 3. Variation of the serum level of IL-27 in relation to lupus nephritis.

Serum IL-27 (pg/ml)	Patients with Lupus nephritis n (%)=41 (82%)	Patients without proved lupus nephritis n (%)= 9 (18%)	Z	P
Median	180	160	176	0.84

IL: Interleukin, n: number, %: percentage, P > 0.05: non significant

Table 4. Comparison between histological classes of lupus nephritis as regard serum IL-27.

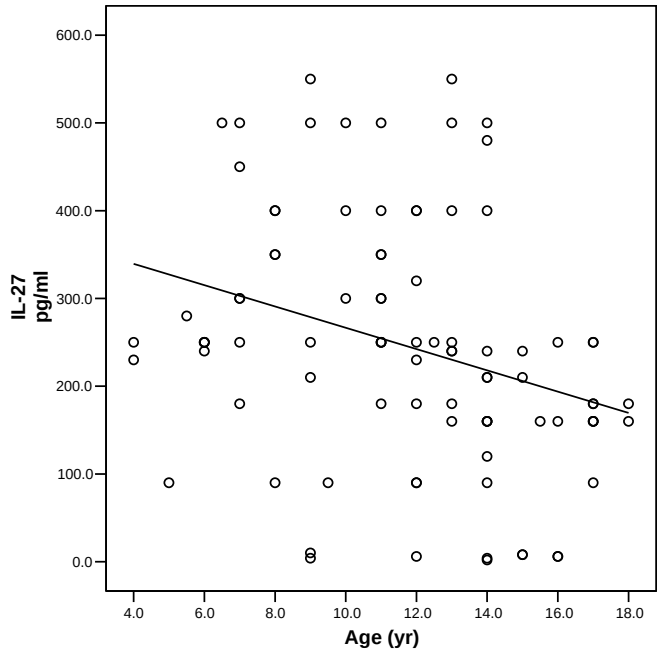
Serum IL-27 (pg/ml)	Patients with LN class II n (%)=22 (54%)	Patients with LN class III-IV n (%)= 18 (44%)	t	P
Mean ± SD	169.8 ± 86.8	154.2 ± 123.2	0. 47	0.64

IL: Interleukin, LN: Lupus nephritis, n: number, %: percentage, P > 0.05: non significant

Table 5. Variation of disease damage in terms of SDI between the studied patients with normal serum IL-27 and those with decreased serum IL-27.

	Normal serum IL-27 n(%)=33(66%)	Decreased serum IL-27 n(%)=17(34%)	z	p
SDI				
Median	0	0	-2.46	0.01
Range	0-0	0-2		

IL: Interleukin, n: number, %: percentage, SDI: Systemic lupus international collaborating clinics/American college of rheumatology damage index, P <0.05: significant.



r=-0.3, p=0.003

Figure 1. A highly significant negative correlation between serum IL-27 and the age of the studied patients.

DISCUSSION

In this study, the finding of significantly lower serum IL-27 in patients with active or inactive lupus when compared to healthy subjects might support the anti-inflammatory role of IL-27 and its probable failure in terms of decreased level and/or compromised function in SLE. However, lupus activity did not affect significantly serum level of IL-27, where the studied patients with active lupus flare had comparable serum level of IL-27 to those with inactive lupus. Decreased serum IL-27 has been reported among adult SLE patients[6,10].

In this series, serum IL-27 was not affected significantly by the sex or ages of the studied patients. However, ages of patients had significantly negative correlation with serum IL-27, a finding that was absent in healthy subjects perhaps indicating a progressive decline in the level of IL-27 in SLE. This needs to be verified in a larger sample size. In contradiction to our results, previous studies found that there was no significant correlation between age of the studied patients and serum IL-27[6, 10].

Decreased serum IL-27 was not associated with younger age at onset of SLE. Although IL-27 seems to have a role in the altered immune response underlying SLE, it is not the only determinant of the disease phenotype. SLE is characterised by a complex interplay between overactive B-cells, abnormally activated T-cells and antigen presenting cells[11].

In the present study, we did not find a significant association between decreased serum IL-27 and LN. Moreover, serum IL-27 was comparable among patients with different histological classes of LN and decreased serum IL-27 did not reflect abnormalities in renal functions. We suggest that serum IL-27 is not a reliable biomarker of LN. On the other hand, it has been reported that serum IL-27 in adult SLE patients with LN was significantly lower as compared to those without LN. However, the authors found that SLE patients with different histological classes of LN had comparable serum IL-27[6,10].

It has been reported that urinary IL-27 was higher in patients with LN, and was inversely correlated with SLEDAI. Moreover, it rose in patients with complete response to treatment[12]. Also, a previous study reported that microarray analysis of glomerular gene expression in murine LN showed a high level of EBI-3, a subunit of IL-27[13]. Several explanations were put for this paradoxical pattern of change in IL-27 expression: First, it might be that IL-27 is produced within the kidney where it exerts local effects. Secondly, it is also possible that urine and serum levels of IL-27 are not correlated. Lastly, some studies looked at cells deriving from renal inflammation/capillary leak, whereas other studies looked at circulating levels. Therefore, further studies are needed to clarify the role of IL-27 in human SLE [6].

In this series, the studied immunological markers (C3, anti-dsDNA, lupus anticoagulants and anti-cardiolipin Abs) were not affected by the serum level of IL-27. Furthermore, we did not find a significant association between serum IL-27 and SLEDAI, suggesting that serum IL-27 albeit decreased in lupus, it does not mirror the activity of the disease. Previous studies reported that there was no significant correlation between serum level of IL-27 and the immunological profile of the adult SLE patients with no significant difference between less active and more active SLE patients[6, 10].

Due to the paucity of patients with other organ involvements in the studied sample, the relationship of serum IL-27 with other SLE manifestations could not be demonstrated in this study. However, a previous study reported that among CNS manifestations, serum IL-27 was significantly lower in patients with psychosis and there was no significant correlation between serum IL-27 and mucocutaneous manifestations, serositis and arthritis[10].

In this series, patients with decreased serum IL-27 had higher SDI as compared to those with normal serum IL-27. This might reflect a prognostic value for serum Il-27 in spite of being a poor indicator of SLE activity. A previous study on adult lupus patients, failed to demonstrate a significant correlation between the serum level of IL-27 and end organ damage such as hypertension and diabetes mellitus[10].

We found that serum IL-27 was not affected significantly by the immunosuppressive therapy given to the studied patients. Accordingly, we propose that the low levels of serum IL-27 could be an inherent defect in SLE patients. A previous study also failed to demonstrate a significant correlation between serum level of IL-27 and the cumulative doses of steroids and cyclophosphamide, while, it proved the existence of a significant negative correlation with the cumulative dose of azathioprine and attributed this to the need of higher doses of medications for longer duration in lupus flare than in remission[10]. However, this possibility is weakened by the lack of effect of cyclophosphamide and steroids on serum IL-27 besides the absence of a significant difference in serum IL-27 between patients with active and less active SLE.

In conclusion, serum IL-27 seems to be inherently low in some SLE patients and might have a role in the immune alteration underlying SLE. However, its exact role remains unclear especially with the lack of significant association with SLE laboratory and clinical characteristics. Further studies are needed to evaluate its exact role.

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