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Serum interleukin-33 (IL-33) in children with active systemic lupus erythematosus: A cross-sectional study

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Abstract

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family and is associated with the development of different autoimmune diseases as systemic lupus erythematosus (SLE). So, the purpose of this cross-sectional study was to measure the serum IL-33 in children with SLE (c-SLE) in relation to their SLE disease activity index. This study was conducted upon 50 c-SLE patients in comparison to 50 normal matched children as a control group. Disease activity was assessed according to SLE Disease Activity Index (SLEDAI-2K). Serum IL-33 was measured by an Enzyme-linked immunosorbent assay (ELISA). Serum IL-33 was significantly higher in c-SLE patients (median: 157.47, IQR:64.49-237.57ng/l) than controls (median: 10.9, IQR: 10.04-12.51ng/L) (*P*= 0.001) and negatively correlated with serum C3 and C4 levels. Serum IL-33 levels were significantly higher in high disease activity status (HDAS) patients (SLEDAI-2K ≥ 10) (298.47 ± 78.84ng/l) than lupus low disease activity status (LLDAS) patients (SLEDAI-2K < 10) (112.18 ± 16.23ng/l) (*P*= 0.001). The receiver operating characteristic (ROC) curve analysis revealed that the best cutoff of serum IL-33 level to predict the disease activity was ≥141.3 ng/l with a sensitivity of 93%, a specificity of 90% and accuracy 97%. We concluded that serum IL-33 was higher in c-SLE patients and positively related to the disease activity index so could be used as a disease activity marker.

Keywords: Interleukin-33, c-SLE, Disease activity index, Activity biomarker.

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Introduction

Childhood systemic lupus erythematosus (c-SLE) is an autoimmune inflammatory disease of children ≤ 18 years, it represents about 20% of all SLE patients.[1,2] It is characterized by systemic inflammation and a wide spectrum of circulating autoantibodies due to the dysfunctional immune regulation.[2] The interleukins are produced by T helper (Th) cells, have an important role in the pathogenesis of SLE and

they are considered to be disease biomarkers because their levels vary with disease activity.[3] c-SLE is more aggressive disorder than adult-SLE (a-SLE) with higher prevalence and severity of lupus nephritis (LN) and neuro-lupus so requiring higher doses of systemic corticosteroids and other immunosuppressive drugs.[4, 5] Interleukin-33 (IL-33) was firstly identified by Baekkevold et al., in 2003[6] from the endothelial cells with similarity to DVS27 of canine, which is a fibroblast growth factor, encodes a nuclear protein that involved in many inflammatory events and considered a novel IL-1 family member.[6, 7] Its target cells are Th-2, basophils, dendritic cells, mast cells, macrophages, and natural killer cells through its suppression of tumorigenicity 2 (ST2) receptors,[8, 9] so, it plays an important role in both innate and adaptive immunity and has a role in many inflammatory and allergic disorders such as atopic dermatitis and asthma.[10, 11] IL-33 is implicated in many autoimmune disorders such as rheumatoid arthritis but its role in SLE is still unclear.[12] To date, serum IL-33 levels have not been investigated in c-SLE. Thus, we aimed to measure the serum IL-33 in children with SLE (c-SLE) in relation to their SLE disease activity index.

Materials and Methods

Study design

This cross-sectional study was conducted on 50 children and adolescents with SLE, below age of 18 years [44 females (88%) and 6 males (12%)]. They were diagnosed according to Systemic Lupus International Collaborating Clinics Classification (SLICC) criteria[13] at initial disease presentation before starting treatment. All study patients were enrolled from the Pediatric, Dermatology and Venereology Departments, Tanta University Hospitals, Egypt during the period between January 2019 and December 2020 and were compared to 50 matched normal children [45 females (90%) and 5 males (10%)]. Patients who had other autoimmune diseases e.g., rheumatoid arthritis, asthma, allergies, obesity, and chronic lung diseases were excluded.

Ethical Approval

The study protocol was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Tanta University (approval no 33146, dated 5-2019). An informed consent was obtained from parents of participants in the study. Willing to provide a blood sample was considered the subject assent.

Laboratory investigations

All study participants were subjected to history taking, clinical examination: thorough general and dermatological examination and laboratory investigations. Data for routine laboratory examination were obtained from patient's records. Serum samples from control subjects were processed similarly. Routine laboratory examination included: complete blood count, 24hr proteinuria, erythrocyte sedimentation rate (ESR), blood urea nitrogen (BUN), creatinine, antinuclear anti-bodies (ANA), anti-double-stranded DNA (anti-dsDNA), complements C3 and C4.

Renal biopsies were done for 33 patients (66%) who met one of the following criteria: increasing serum creatinine without alternative causes, proteinuria of ≥ 1g/24hr, proteinuria ≥ 0.5 g/24hr with hematuria or cellular casts. Renal biopsies were evaluated according to the International Society of Nephrology Renal Pathology Society (ISN/RPS) grading system. [14] Disease activity was assessed according to SLE Disease Activity Index (SLEDAI-2K).[15] High disease activity status (HDAS) of patients was defined as attainment of SLEDAI-2K ≥ 10 and lupus low disease activity status (LLDAS) defined as attainment of SLEDAI-2K < 10. [16]

Sample collection and IL-33 assessment

Venous blood samples (3ml) were collected from study subjects in sterile tubes and allowed to clot, then centrifuged at 447 xg for 10 min. Specimens were capped and stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time were stored at -20°C prior to assay to avoid loss of bioactive human IL-33. Repeated freeze cycles were avoided. Prior to assay, the frozen sample was brought to room temperature slowly and mixed gently. Serum IL-33 levels were assessed by an enzyme-linked

immunosorbent assay technique (ELISA) using a commercially available kit (provided by Biokit, A Werfen Company, Barcelona, Spain), according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was done by SPSS version 27 (IBM©, Chicago, IL, USA). Shapiro-Wilks's test and histograms were used to evaluate the normality of the distribution of data. Quantitative parametric data were presented as mean and standard deviation (Mean ± SD), and were analyzed by unpaired student t-test. Quantitative non-parametric data were presented as median and interquartile range (Median ± SD), and were analyzed by Mann Whitney-test. Qualitative variables were presented as frequency and percentage (n, %) and were analyzed by Chi-square test. Data correlations were assessed by Spearman rank correlation coefficient. A receiver operating characteristic (ROC) curve was drawn to assess the value of serum IL-33 to predict the disease activity in children with SLE. A *P* value < 0.05 was considered statistically significant.

Results

The clinical and the laboratory data of the studied c-SLE patients and controls are shown in (Table1). Their ages ranged between 9-17 years, (Mean ± SD; 13.64±2.48) and 8-18 years (13.76±2.15), respectively. Female/male ratio was 7.3/1 in c-SLE patients. Fever (82%), hematological (76%), mucocutaneous (68%) and renal manifestations (66%) were the commonest initial disease presentations. ANA was positive in all studied patients (100%), anti-ds DNA was positive in 92% of patients.

Thirty-three patients (66%) had renal affection at initial disease presentation, 24hr proteinuria median (IQR) levels were: 651(2337-2772) mg, the histopathological results of their renal biopsies were LN Class II (4%), LN class III (40%), LN class IV (20%), and only 2% had LN Class V.

Table 1. Clinical and laboratory data of c-SLE patients and normal controls.

	c-SLE	Controls	*P*-value
Age (years)			
Range	9 - 17	8 - 18	NS
Mean ±SD	13.64 ± 2.48	13.76 ± 2.15	
Sex n (%)			
Female	44 (88%)	45 (90%)	
Male	6 (12%)	5 (10%)	NS
Female/male ratio	7.3/1	9/1	
Clinical manifestations (n %)			
Fever	41 (82%)		
Hematological	38 (76%)		
Renal	33 (66%)		
Cutaneous rash	22 (44%)		
Arthritis	14 (28%)		
Mucosal ulcer	8 (16%)	-	-
Alopecia	4 (8%)		
Vasculitis	2 (4%)		
Pericarditis	2 (4%)		
Myositis	1 (2%)		
Pleurisy	1(2%)		
Neurological	1(2%)		
Hb (g/dl) (Mean ±SD)	9.73 ± 1.45	12.74 ± 1.32	0.001
PLT (x10^3/ml) (Mean ±SD)	129.87 ± 47.80	276.30 ± 51.35	0.001
TLC (x10^3/ml) (Mean ±SD)	3.45 ± 0.84	6.76 ± 2.93	0.001
1st hr ESR (mm) Median (IQR)	45.5 (31.75-61.25)	12 (5-20)	0.001
Creatinine (mg/dl) (Mean ±SD)	0.93 ± 0.28	0.56 ± 0.15	0.006

Table 1. Continued.

	c-SLE	Controls	*P*-value
BUN (mg/dl) (Mean ±SD)	18.70 ± 4.76	14.60 ± 2.93	0.003
Albumin (g/dl) (Mean ±SD)	3.03 ± 0.76	4.23 ± 0.58	0.001
24hr proteinuria (mg) Median (IQR)	651 (2337-2772)	118 (70-135)	0.001
C3 (mg %) (Median (IQR)	61 (52-98)	123 (95-156)	0.001
C4 (mg %) (Median (IQR)	8 (5.3-21.2)	24 (15.6-34)	0.001
Positive ANA n (%)	50 (100%)	-	0.001
Anti-dsDNA (IU/ml) Median (IQR)	114 (60-195)	18 (10-35)	0.001
Positive Anti-dsDNA (n %)	46 (92%)	-	
SLEDAI-2K Median (IQR)	14 (8 - 22)	-	0.001
LN classes: n (%)			
LN Class II	2 (4%)		
LN Class III	20(40%)		
LN Class IV	10 (20%)	-	
LN Class V	1 (2%)		
IL-33 (ng/l) Median (IQR)	157.47 (64.49 - 237.57)	10.9 (10.04 - 12.51)	0.001

ESR: Erythrocyte sedimentation rate, Hb: Hemoglobulin. TLC: Total leucocytic count, Plt: Platelets, C3, C4: Complement 3, 4, ANA: Antinuclear antibody, Anti-ds DNA: Anti-double strand DNA antibody SLEDAI: Systemic lupus erythematosus disease activity index, LN: Lupus nephritis, IL-33: Interleukin 33, $P > 0.05$ is not significant (NS).

Serum IL-33 levels were significantly higher in patients with median (IQR) [157.47 (64.49 - 237.57) ng/l] than in controls [10.9 (10.04 - 12.51) ng/l], (P<0.01). Serum IL-33 levels were significantly higher in HDAS patients (SLEDAI-2K ≥ 10) (298.47 ± 78.84ng/l) than in LLDAS patients (SLEDAI-2K < 10) (112.18 ± 16.23ng/l) (P=0.001) (Table 2, Figure 1).

Table 2. Serum IL-33 level in HDAS and LLDAS patients.

	HDAS patients (SLEDAI-2K ≥ 10) (n=28)	LLDAS patients (SLEDAI-2K < 10) (n=22)	*P*-value
Serum IL-33 (ng/l) Mean ±SD	298.47 ± 78.84	112.18 ± 16.23	0.001

HDAS: High disease activity status; LLDAS: Lupus low disease activity status. $P \leq 0.05$ is significant.

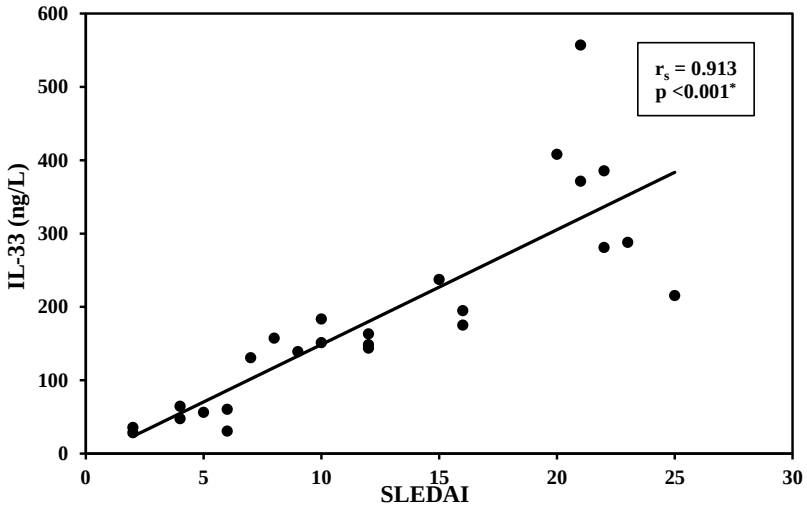

Figure 1. Correlation between serum IL-33 and SLEDAI-2K in c-SLE patients.

Serum IL-33 level was higher in proliferative LN classes III and IV with significant positive correlation with 24hr proteinuria (*P*= 0.003) (Table 3 and figure 2).

Table 3. Relation between serum IL-33 and LN in c-SLE patients

	Lupus Nephritis (LN)			*P* value
	No. LN (n= 17)	LN classes II, III (n= 22)	LN classes IV, V (n= 11)	
Serum IL-33 (ng/l)				
Median	62.43	163.2	259.27	0.003
(IQR)	37.69-118.5	78.3-348.2	185.2-467.5	

LN: Lupus nephritis. $P \leq 0.05$ is significant.

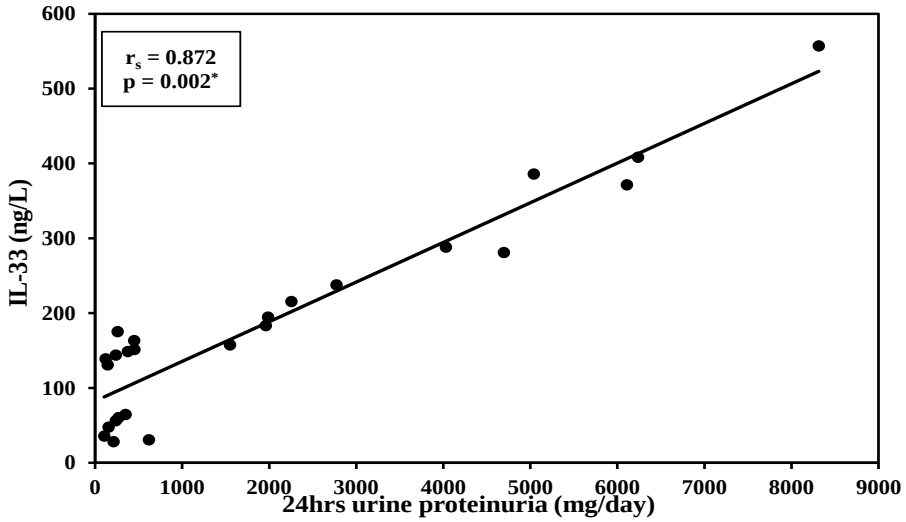

Figure 2. Correlation between serum IL-33 and 24hr proteinuria in c-SLE patients.

Serum IL-33 level was negatively correlated with serum C3 and C4 levels (*P* < 0.05) (Figures 3 & 4). The ROC curve analysis showed that the best cutoff serum IL-33 level to predict disease activity was ≥141.3 ng/l with a sensitivity of 93%, a specificity of 90% and accuracy 97% (Figure 5).

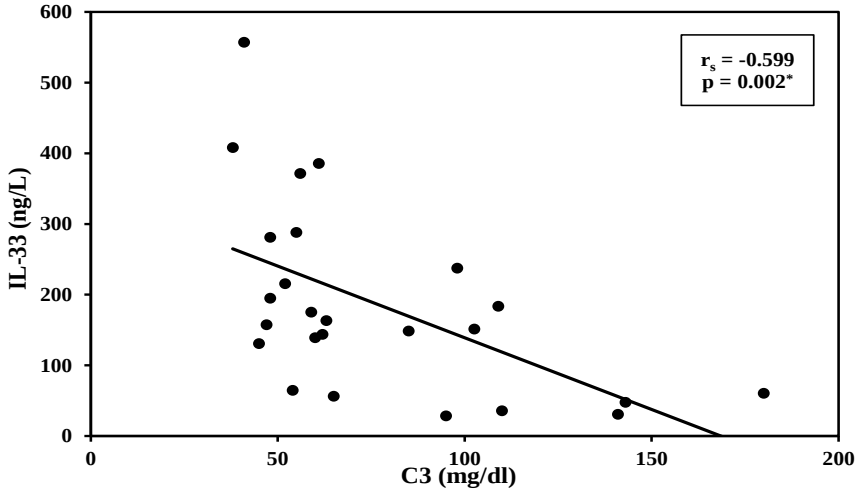

Figure 3. Correlation between serum IL-33 and C3 in c-SLE patients.

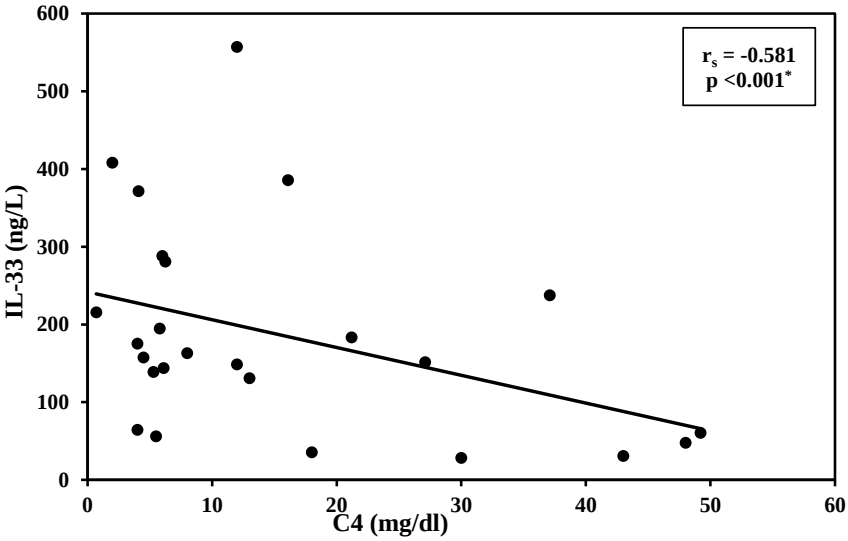

Figure 4. Correlation between serum IL-33 and C4 in c-SLE patients.

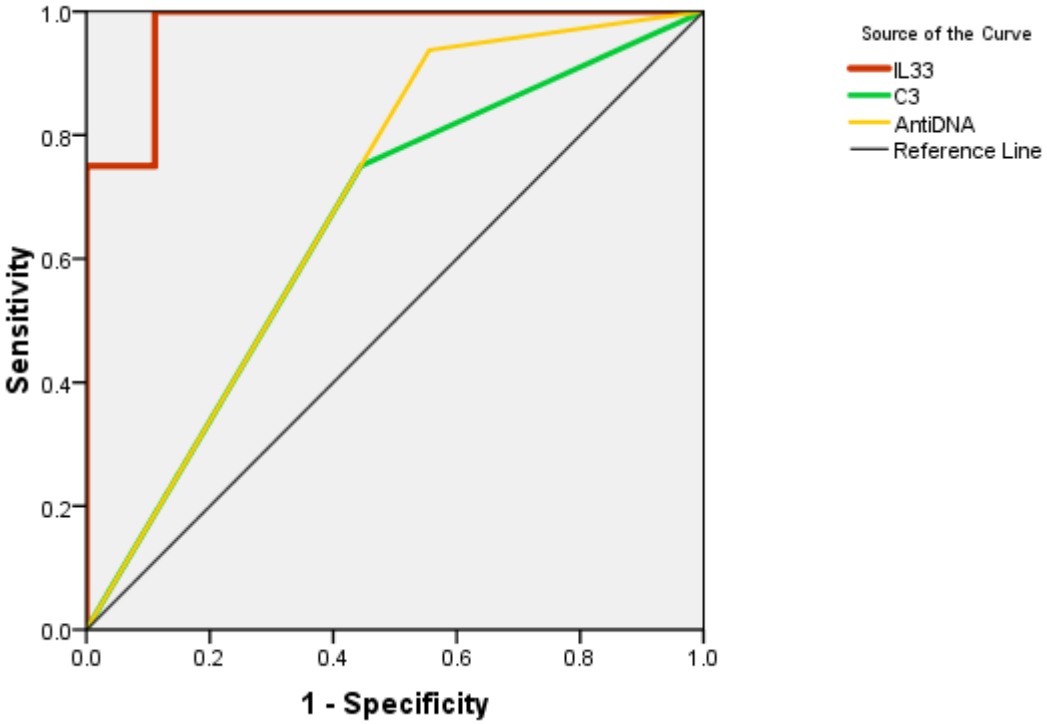

Figure 5. Receiver operating characteristic (ROC) curve analysis of serum IL-33.

Discussion

Interleukin-33 is a pleiotropic cytokine belongs to the inflammatory IL-1 family activating Th-2 cells, and so involved in many disorders such as infection, cancer and autoimmune diseases (rheumatoid arthritis RA, systemic sclerosis, inflammatory bowel diseases, and SLE).[17] Serum IL-33 levels of the studied c-SLE patients were significantly higher than the controls. In line with this study findings, a study by Yang et al., 2011,[18] found that serum IL-33 levels were significantly higher in a-SLE patients than in their study controls. Also, GUO et al., 2016[19] demonstrated the same results in Chinese a-SLE patients. In contrast, Mok et al., 2010[20] reported that the majority of their a-SLE patients had low IL-33 levels with no difference between patients

and controls. Such discrepancy in study findings is probably due to the detection efficacy of the ELISA kits used or to the multiple roles of IL-33 in the disease and different included sample characteristics.[21]

In the current study, there was a significant negative correlation between IL-33 and C3 and C4. This may be explained by consumption of complement system in immune complexes deposition during active phase of SLE with simultaneous elevation of IL-33 during disease activity. On the contrary, a previous study done by Yang et al., 2011[18] observed that serum IL-33 concentrations did not significantly correlate with either the level of C3 or C4.

The current study showed a significant positive correlation between serum IL-33 and severity of c-SLE as evaluated by SLEDAI-2K score. This observation agreed with findings of two previous studies, by XU et al., 2016[22] and by Zhu et al., 2019[23] who reported close correlation between serum IL-33 and SLEDAI. Thus, serum IL-33 may help to identify SLE patients with active and severe disease.

According to renal affection in our studied patients, serum IL-33 had a significant positive correlation with 24hr proteinuria and was significantly higher in LN classes III and IV in comparison to patients without LN. The best cutoff of serum IL-33 level to predict disease activity was ≥141.3 ng/L with a sensitivity of 93%, a specificity of 90% and accuracy 97%. To further explore IL-33 effect, a study by Li et al., 2014[10] found that the administration of neutralizing antibodies against IL-33 in MRL/lpr lupus mice reduced serum anti-dsDNA level and immune complex deposition in their blood vessels and kidneys. This protective effect was associated with increase T-regulatory cells (Tregs), reduction of Th17 cells and pro-inflammatory factors. Finally, a study by Dong et al., found two polymorphisms of the IL-33 gene (rs1929992-G and rs1891385- C) that were associated with a higher susceptibility to SLE.[24]

In conclusion, our study indicated that serum IL-33 was higher in c-SLE patients and positively related to the disease activity index, therefore assessment of IL-33 levels could be used as a disease activity marker.

Author Contributions

RA, HA and WM, material preparation and data collection. TA and DH, data analysis and revision. HA, the first draft of the manuscript was written. All authors contributed to the study conception, design and revision. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Tanta University (approval no 33146, dated 5-2019).

Informed consent

An informed consent was obtained from parents of participants in the study. Willing to provide a blood sample was considered subject assent.

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