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Original Article

Interleukin-6 and disease activity in childhood systemic lupus erythematosus

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Abstract

Background Systemic lupus erythematosus (SLE) is a complex disease with various manifestations. Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biological activities which plays an important role in immune regulation and inflammation. Serum level of IL-6 may be used as a parameter of disease activity, especially in pediatric SLE patients with mild disease activity or total remission with conflicting clinical manifestations and *Systemic Lupus Erythematosus Disease Activity Index* (SLEDAI) scores.

Objective To identify the characteristics of serum IL-6 concentration in pediatric SLE with mild disease activities and total remission.

Methods This case-control study was performed at the Allergy-Immunology Outpatient Clinic, Department of Child Health Dr. Cipto Mangunkusumo Hospital, Jakarta and Dr. Sardjito Hospital, Yogyakarta. Serum IL-6 concentration and disease activity were assessed in all pediatric SLE patients aged 1-18 years. Disease activity was assessed with SLEDAI scores and serum level of IL-6 was measured by enzyme-linked immunosorbent assay.

Results Among 60 subjects included in this study, 30 subjects with mild activities were in the case group and 30 subjects with total remissions were in the control group. There was no difference in serum IL-6 concentration between the case and control group (OR 0.483; 95%CI 0.041to 5.628; P=0.500). In this study, 2 subjects with urinary tract infection had high serum IL-6 concentrations.

Conclusion There is no difference in serum IL-6 concentration between pediatric SLE patients with mild disease activities compared to total remissions. **[Paediatr Indones. 2023;63:456-63; DOI: https://doi.org/10.14238/pi63.6.2023.456-63**].

Keywords: *systemic lupus erythematosus; interleukin-6; disease activity; systemic lupus erythematosus disease activity index; SLEDAI*

Systemic lupus erythematosus (SLE) is a complex disease with various manifestations.[1] Pathogenesis of SLE is complex and multifactorial, involving environmental, hormonal, and immunological factors in individuals with genetic predisposition.[2,3] These factors contribute to the damage of reactive B cell tolerance. SLE patients with childhood onset, diagnosed under 18 years of age, constitute 15-20% of all SLE cases.[2] Childhood onset SLE is rare, with an incidence of 0.3-0.9 over 100,000 children in a year.[4]

Systemic lupus erythematosus is associated with immune hyperactivity, which can be detected as an enhancement of cytokine concentration aligned with receptor upregulation by hematopoietic cells.[5] Cytokine is an important mediator in intercellular communication and immune-cell interaction within the immune response. In SLE cases, several cytokines are involved in immune dysregulation and local inflammation which impact tissue and organ damage.[6]

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Interleukin 6 (IL-6) is a pleiotropic cytokine that plays an important role in immune regulation and inflammation. Interleukin 6 induces B lymphocyte differentiation to become an antibody and T cell differentiation to become an effector cell.[6] In SLE disease, B lymphocytes express IL-6R (receptor) spontaneously, produce large amounts of IL-6, and increase B lymphocyte activation and auto-antibody production.[7] Patient with active SLE disease shows an increase in serum IL-6 concentration. In comparison to healthy control, SLE patients have significantly higher IL-6 concentrations.[7,8] The relationship between IL-6 and SLE was also demonstrated in the SLE model mouse in which inhibition of IL-6 improved the condition of SLE in all experimental animals tested. IL-6 has shown an important role in B cell hyperreactivity and SLE immunopathology in humans and may have a direct role in mediating tissue damage.[9]

To date, no study has investigated IL-6 concentration in pediatric SLE patients in Indonesia. Serum level of IL-6 may be used as a parameter for disease activity. This is especially true in cases where different clinical manifestations and *Systemic Lupus Erythematosus Disease Activity Index* (SLEDAI) score is found, furthermore among pediatric SLE patients with mild disease activities and total remissions. This study aimed to identify the characteristics of serum IL-6 concentration in pediatric SLE with mild activities and total remissions based on clinical manifestation and laboratory parameters.

Method

This case-control study was performed at an Outpatient Clinic of Allergy-Immunology, Department of Child Health Dr. Cipto Mangunkusumo Hospital, Jakarta and Dr. Sardjito Hospital, Yogyakarta, in March-June 2019. The study participants were SLE patients diagnosed at < 18 years old based on *1997 American College of Rheumatology* (ACR) criteria who were routinely followed up at the outpatient clinic and were willing to participate in this study. Serum IL-6 concentration and disease activity were then assessed. Disease activity was assessed using SLEDAI scores and serum level of IL-6 was measured by enzyme-linked immunosorbent assay. Participants were divided into a case group and a control group. The case group was SLE patients with mild disease activity (based on SLEDAI score 1-5), with a good clinical condition, low complement concentration or high ds-DNA concentration. The control group was patients with total remission (good clinical and laboratory value, SLEDAI score 0). Exclusion criteria were newly diagnosed patients (less than 3 months), patients with severe systemic infection conditions, or patients diagnosed with other autoimmune diseases that mimicked SLE, such as mixed connective tissue disease (MCTD).

The study data were analyzed using *SPSS program version 22.0*. Differences in IL-6 serum level based on disease activity were analyzed with an independent T-test for normal distribution or Mann-Whitney test for non-normal distribution. After differences in IL-6 serum levels were discovered, comparisons between the case and control groups were then analyzed using Chi-square (X^2) to obtain an odd ratio value (OR). A P value of <0.05 was considered statistically significant.

This study protocol was approved by the ethical committee from both the Faculty of Medicine, Universitas Gadjah Mada and the Faculty of Medicine, Universitas Indonesia.

Results

There were 72 and 89 childhood-onset SLE patients undergoing therapy at the outpatient clinic, Allergy-Immunology Division of Dr. Sardjito Hospital and Dr. Cipto Mangunkusumo Hospital, respectively. Sixty-three eligible subjects from both hospitals met the inclusion criteria. Three patients were then excluded due to lysed blood samples. The case and control groups each consisted of 30 subjects. In total, 60 patients from both hospitals were included as study subjects.

The baseline characteristics of this study are listed in **Table 1**. The average age of SLE diagnosis was 13 (range 9-17) years in the case group and 12 (range 4-15) years in the control group. The youngest age of SLE was diagnosed at 4 years in the control group. The subject proportion who underwent menarche before diagnosis was higher in the case group (16/30 subjects) compared to the control group,

although it was not statistically significant.

Figure 1 shows the two most common clinical manifestations (based on ACR criteria) were malar rash and non-erosive arthritis in both case and control groups. While the most common laboratory parameter was immunology and hematology disorder, with positive ANA present in both groups. **Table 2** further reveals that hematology disorder remained the most common manifestation in laboratory parameters within the last 3 months, with a higher incidence in the case group (5/30 *vs.* 3/30).

Steroid therapy was used in all subjects from the initial diagnosis to the last 3 months, both in the case and control groups. The type of steroid therapy that was widely used was oral methylprednisolone, from the start of diagnosis (22/30 *vs.* 27/30) to the last 3 months (28/30 *vs.* 27/30), whereas prednisone steroid therapy was only used in 5 subjects since the start of diagnosis and 2 subjects in the last 3 months. High doses of intravenous methylprednisolone were administered to 25 subjects from the time of diagnosis (12/30 *vs.* 13/30). Meanwhile, this therapy was used

Table 1. Basic characteristics of study subjects

Characteristics	Cases (n=30)	Control (n=30)	P value
Mean age (SD), years	15.47 (2.25)	13.50 (2.88)	0.005*
Median age at diagnosis (range), years	13 (9-17)	12 (4-15)	0.048**
Sex/female n	27	26	0.500***
Menarche history, n	16	13	0.438****
Nutritional status, n			
Underweight	6	4	0.129*****
Normal	20	17	
Overweight	3	5	
Obesitas	1	4	

*Analysis with independent test; **analysis with Mann Whitneytest; ***analysis with Fisher exact test; ****analysis with Chi-square test; *****analysis with Kruskal Wallis test.

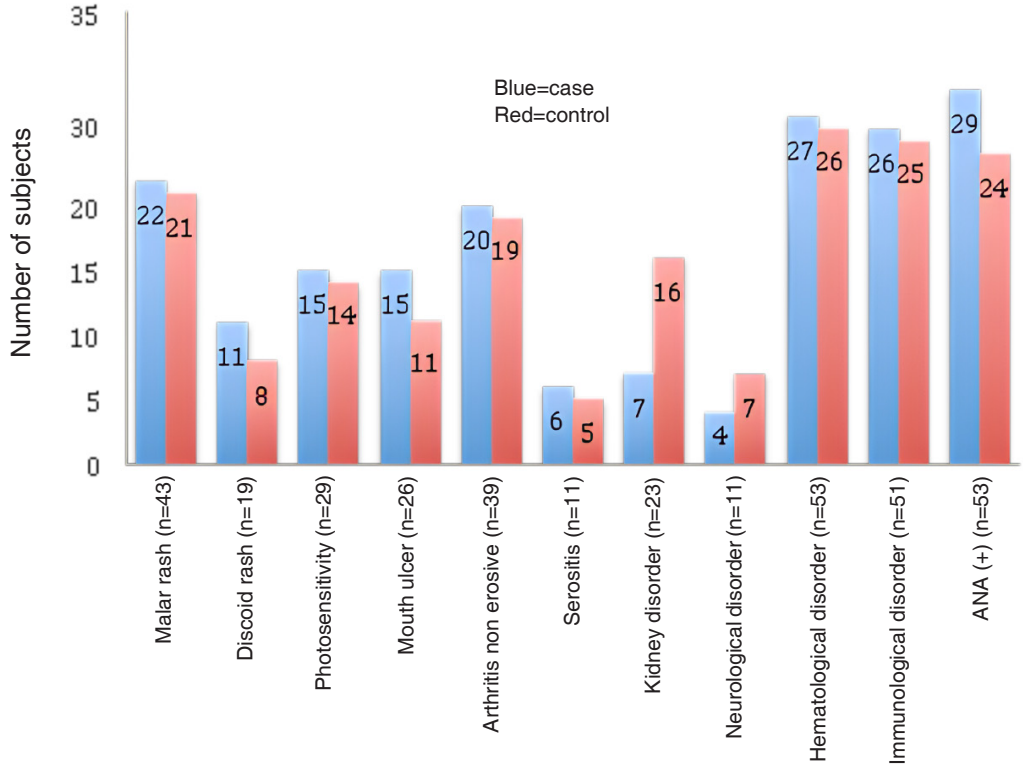

Figure 1. Clinical manifestations in SLE diagnosis (ACR Criteria)

Table 2. Clinical manifestations and laboratory profiles in the last three months

Clinical manifestations and laboratory profiles, n (%)	Cases (n=30)	Control (n=30)
Hematology	5	3
Leukopenia	4	3
Thrombocytopenia	1	0
Nephrology	1	0
Neurology	0	0

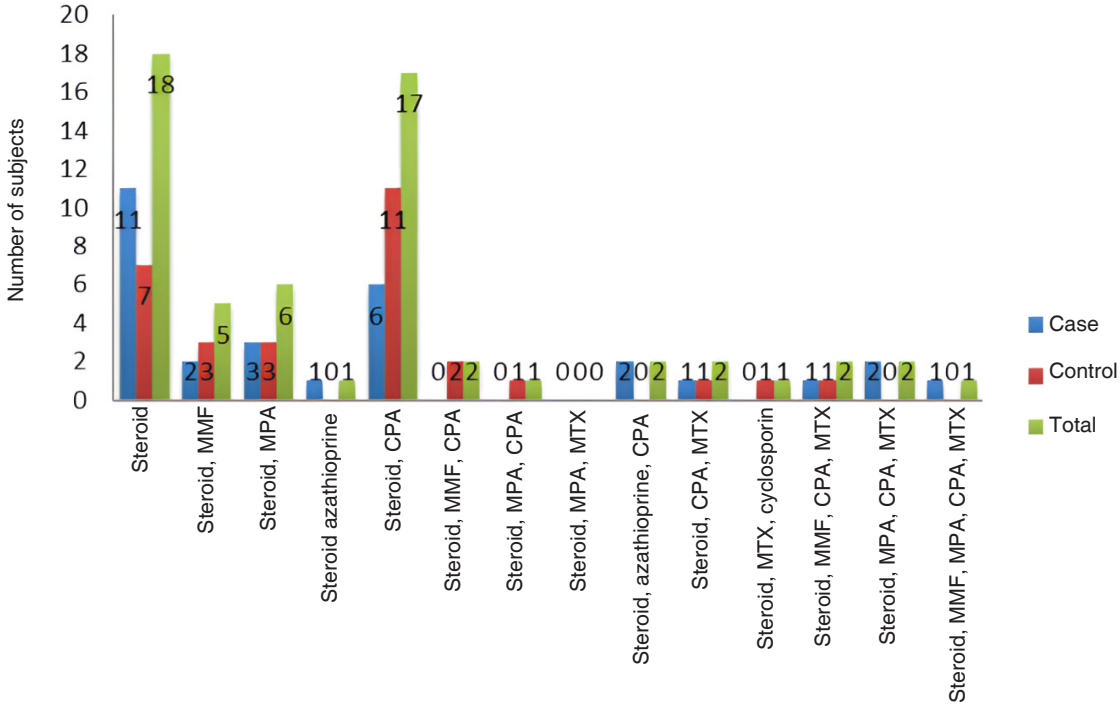

Figure 2. Proportion of single and combined therapy
(MMF=mycophenolate mofetil, MMA=mycophenolic acid, CPA=cyclophosphamide, MTX=methotrexate)

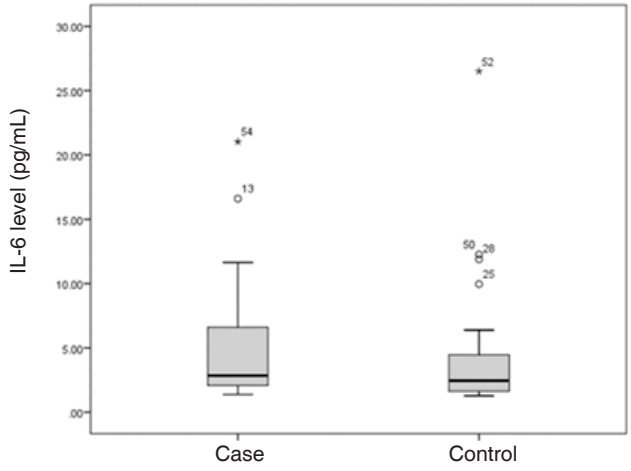

Figure 3. IL-6 profiles in case and control group

on 6 subjects in the last 3 months, 2/30 of the case group and 4/30 of the control group.

Single steroid therapy was found more in the case group than in the control group (**Figure 2**). The most widely used combination therapies were steroid-MPA (mycophenolic acid), steroid-MMF (mycophenolate mofetil), and steroid-CPA (cyclophosphamide).

Figure 3 shows that the average IL-6 level in the case group was higher than the control group with a wide median range. The result from **Table 3** shows that there was no significant difference in high IL-6 levels between the case group and the control group, (OR 0.483; 95%CI 0.041 to 5.628; P=0.500).

In this study, high IL-6 levels were not found in the hematology, nephrology, and neurology manifestations (**Table 4**). **Table 5** shows one of the case group's subjects and two of the control group's subjects with high IL-6 levels. Two subjects were diagnosed with urinary tract infection for the last 3 months.

Discussion

Results of this study indicated that the mean age, age at diagnosis, female sex, and nutritional status were consistent with the results of previous studies. The majority of SLE patients are women of reproductive age. In this study, the average age of the mild activity group was 15.47 (SD 2.25) years, while the average age of total remission was 13.5 (SB 2.28) years . This was consistent with other studies, which stated that the median age of onset of SLE in children was between 11-12 years of age.[4] The mean age at SLE diagnosis was 13 (range 9-17) years in mild SLE activity and 12 (range 4-15) years, respectively, in total remission.

Table 3. Comparison analysis of IL-6 level

IL-6 levels	Cases (n=30)	Control (n=30)	OR (95%CI)	P value
High (>12.5 pg/mL)	1	2	0.483 (0.041 to 5.628)	0.500
Normal (< 12.5 pg/mL)	29	28		

Table 4. Characteristics of High IL-6 Subjects

Characteristics	High IL-6 subjects		
	1st Subject	2nd Subject	3rd Subject
Age, years	16	15	16
Sex	Female	Female	Female
Group	Control	Control	Cases
Clinical manifestations last 3 months (based on SLEDAI)			
Hematology	(-)	(-)	(-)
Leukopenia	(-)	(-)	(-)
Thrombocytopenia	(-)	(-)	(-)
Nephrology	(-)	(-)	(-)
Neurology	(-)	(-)	(-)
Abnormal laboratory	Hb (10.1 mg/dL)	Urine bacteria (295.30/µL)	Urine bacteria (+)
C3/C4 ratio	167 (N) / 27 (N)	156 (N) / 34 (N)	52 (↓) / 13 (↓)
Anti ds DNA level, IU/mL	8.20 (N)	10.70 (N)	273.10 (↑)

Table 5. IL-6 proportion based on clinical manifestations and laboratory profiles

Clinical manifestations and laboratory profiles	IL-6	
	High, n (%)	Low, n (%)
Hematology	0 (0.0)	8 (14.0)
Nephrology	0 (0.0)	1 (1.8)
Neurology	0 (0.0)	0 (0.0)

Mean age at diagnosis found in most cohort studies varied between 24-32 years of age in adults and 12-17 years of age in children.[10] The maximum age used to define children's SLE was 16 years.[11]

Systemic lupus erythematous is more commonly found in women than men. In our study, most of the subjects were women (88.3%). Another study reported that 90% or more of SLE patients were women. As in adult-onset SLE, approximately 80% of children's SLE were female.[4] Another study found that the incidence and prevalence of SLE was 1/10 for males compared to females.[12]

In the case group, 27 patients had experienced menarche and 26 patients in the control group. Since 90% of SLE patients are women, SLE likely involves female hormones, but a protective role for male hormones or genes on the X chromosome is also possible.[13] In a previous study, menstrual cycle abnormalities and hormonal imbalances, such as decreased progesterone levels and hyperprolactinemia, were found to be significant against high SLEDAI scores ($P<0.05$, $P=0.001$, $P<0.05$, respectively). The study concluded that disease activity was a major factor associated with menstrual cycle abnormalities and changes in ovarian function.[14] A study in Brazil analyzed how gonadal function and age at menarche in SLE patients correlated with clinical manifestations, SLEDAI parameters, and treatment. The mean age at menarche [13.5 (SD 1.4) years] was older than the 2,578 healthy adolescents in Brazil [12.5 (SD 1.3) years; $P<0.0001$]. The results of this study did not indicate a delay in menarche in the two groups.[15]

In another study in Brazil, it was observed that more than 50% of subjects were in normal nutritional status. Obesity, as classified by nutritional status, was found in 5 subjects, constituting 13.3% of the total remission. This study also found that overweight was found in SLE patients in Brazil and was associated with several risk factors, such as heart disease, and poor prognosis.[16]

The diagnosis of SLE can be confirmed if 4 out of 11 criteria are obtained based on the revised classification of the *American College of Rheumatology* (ACR) in 1982. These criteria include clinical features and laboratory results.[10] This criterion has a sensitivity of 85% and a specificity of 95%. In this study, the most common clinical manifestations at diagnosis (based on ACR criteria) were positive ANA, hematological disorders, and immunological disorders. Serositis and neurological disorders wererare clinical manifestations in this study.

In this study, it was found that steroid therapy was used in 100% of subjects from the initial diagnosis up to the last 3 months, both in the case and control groups. The most widely used steroid was oral methylprednisolone from the initial diagnosis (22/30 *vs.* 27/30) to the last 3 months (28/30 *vs.* 27/30), whereas prednisone steroid therapy was rarely used. High doses of intravenous methylprednisolone were used in 25 subjects from the start of the diagnosis, namely 12/30 of the cases group and 13/30 of the control groups. Oral and intravenous corticosteroids remain the most effective therapeutic drugs for rapid control of the disease.[4] Pediatric SLE patients in 2 Canadian tertiary hospitals were prescribed oral corticosteroids more often than adults (97% of 67 pediatric SLE patients *vs.* 70% of 131 adult patients). Children with SLE received intravenous methylprednisolone therapy almost three times as often as adults.[1]

The mean IL-6 level in the mild activity group was not significantly different from the total remission group (2.84 pg/mL *vs.* 2.45 pg/mL, $P=0.137$). The average IL-6 level from this study was lower than other studies, which stated that the mean IL-6 level serum of SLE patients was 68.1 (SD 68.0) pg/mL, which is 4.8 times higher than healthy individuals [14.16 (SD 5.83) pg/mL] and it was statistically significant ($P<0.0001$). Mean IL-6 levels were significantly higher among active SLE [74.9 (SD 68.5) pg/mL] compared with inactive SLE [19.0 (SD 3.8) pg/mL], $P=0.0036$.[8]

The results of this study showed that there was no difference in the proportion between high IL-6 levels in the mild activity group and total remission group with OR 2.07 (95%CI 0.178 to 24.148; $P=1.00$). This result differed from previous studies because this was the first study that compared mild activity and total remission subjects. Meanwhile, previous studies have mostly compared active SLE with inactive or healthy individuals as the control group. A study reported that patients with active disease had higher levels of IL-6 and IL-10 than patients with inactive disease ($P=0.001$ and $P=0.014$, respectively) and the control group (both $P<0.001$). The levels of IL-6 ($P=0.022$), IL-10 ($P=0.013$),

and IL-17A (P=0.041) were significantly higher during active than inactive disease.[2] Likewise, other studies stated that serum IL-6 concentrations were increased in SLE patients compared to control groups and correlated with SLEDAI scores.[6,8,9] Active SLE patients had elevated IL-6 levels which in some studies were associated with disease activity or anti-DNA levels.[6] There was a significant positive relationship between serum IL-6 and IL-17 concentrations during the active period (r=0.497, P=0.005) and remission (r=0.662, P <0.001).[17]

This study did not find high IL-6 level in the hematology, nephrology and neurological manifestations in the last 3 months. Another study suggested that elevated serum levels of cytokines (IL-17 and IL-6) were associated with active lupus nephritis and anemia, and were positively correlated with SLEDAI-2k scores (P=0.025 for IL-17 and P<0.001 for IL- 6).[17] The IL-6 and IL-10 serum levels were positively correlated with SLEDAI scores and anti-dsDNA titer but negatively correlated with serum C3 and C4 levels.[18]

Three subjects had high IL-6, levels and two of them had urinary tract infection. A previous study stated that IL-6 is a pleiotropic cytokine produced rapidly and temporarily in response to tissue damage and infection.[19] IL-6 contributes to host defense through stimulation of acute phase response, hematopoiesis, and immune reaction.[20]

In conclusion, there is no different characteristic of IL-6 levels between patients with mild activity SLE and those with total remission SLE. Two subjects with high IL-6 levels suffered urinary tract infection.

Conflict of interest

None declared.

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