Serum Interleukin-2 Level Associated with Treg/Th17 Ratio in Active Systemic Lupus Erythematosus

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is resulted from loss of immunological tolerance. Interleukin-2 (IL-2) plays an important role in loss of tolerance in SLE through its influence on Treg and Th17 balance. This study was conducted to determine the correlation between serum IL-2 level with Treg/Th17 ratio in active SLE.

Methods: This cross-sectional analytic study included thirty newly diagnosed SLE patients. IL-2 level was measured using ELISA, whereas Treg and Th17 expressions were measured using the flow cytometry method, then the ratio was calculated. The Systemic Lupus Activity Measure (SLAM) scores were used to assessed SLE disease activity.

Results: All patients were female with mean age of 31±10 years. Hematological conditions and arthritis were the most prominent clinical manifestations. All patients were in active SLE conditions with a high mean SLAM score of 29±4. Serum IL-2 level was lower in SLE patients compared to healthy subjects (median 7.12 pg/mL vs 49.31 pg/mL, respectively). Treg/Th17 ratio was also lower in SLE patients compared to healthy subjects (median 0.405 vs 0.830, respectively). There was a significant correlation between serum IL-2 level with Treg/Th17 ratio ($r = 0.463$; $P = 0.01$).

Conclusion: Serum IL-2 level is associated with Treg/Th17 ratio in active SLE.

Keywords: SLE, IL-2, Treg/Th17 ratio, disease activity, SLAM.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune systemic disease that involves diverse pathogenesis and various illnesses, from mild to life-threatening [1,2]. Lupus symptoms that appear at any time are very potential to disrupt daily activities and cause many other problems. To achieve optimal health status and high quality of life, SLE patients must be proactive in managing the disease [3]. All changes in conditions must be experienced by patients with SLE both in environmental aspects, such as social support, physical aspects and emotional aspects affecting changes in their life quality [4].

It is known that the pathogenesis of SLE is based on interactions of genetic predisposition, hormonal factors, and environmental triggers. The previous study presented the marker of SLE disease activity might be Midkine serum level and have a role in the pathogenesis of SLE [5]. However, to date, the specific cellular and humoral mechanisms underlying immune dysfunction in SLE have not been fully understood. The main factor in the occurrence of autoimmune

in SLE is the loss of tolerance [6]. SLE pathogenesis is characterized by the absence of self-tolerance, with autoreactive T-cells and the activation of autoreactive B-cell, resulting in pathogenic autoantibodies and tissue damage. Self-antigen tolerance is retained by T cell subpopulations, referred to as Regulatory T (Tregs), which help to control immunological balance, and tolerance loss is an underlying autoimmune pathology [7,8]. Decreased production of IL-2 in SLE is a factor in immune dysregulation that underlies the loss of tolerance in SLE. IL-2 is important in maintaining self-tolerance and inhibit autoimmunity. Low IL-2 contributes to a decrease in the number of Treg cells, which are essential to the regulation of autoreactive T cells expansion [9]. The decrease of IL-2 levels in SLE have been reported in various studies and are thought to play a major role in loss of tolerance[9,10] through their influence on Treg and Th17 balance [11,12].

The reduction in IL-2 development also results in a disruption in cell death induced by activation

induced cell death (AICD) that is necessary to downregulate T cell clone growth [13]. In order to produce the CD8 + T-Cell Effector functions and CD8 + T cell memory development, IL-2 is also necessary. The failure of the CD8+T cytotoxic feature increases the risk for SLE patients of intracellular infection [9]. Increasing the number of Treg cells by IL-2 can be a specific therapeutic strategy to restore self-tolerance to SLE, but functional improvement and abnormal immune responses must be carefully analyzed in SLE patients given the complexity of the pathogenesis and etiology of SLE disease.

Data from various studies on IL-2 in SLE have shown opposing results about the role of IL-2 in SLE. Multiple studies reported a relationship between IL-2 levels and SLE disease activity [14–16]. Whereas, another study reported that IL-2 levels were not associated with SLE disease activity [17]. Various studies on the therapy of low-dose IL-2 in SLE have also been carried out. Low-dose IL-2 therapy increases the population of Treg cells and decreases the number of TFH and Th17 cells, followed the decreased of disease activity in SLE patients [18,19].

Considering the importance of IL-2 in SLE and the limited research data on the role of IL-2 in the mechanism and activity of SLE disease in Indonesia, this study was conducted to determine the correlation between serum IL-2 levels and Treg/Th17 ratio in active SLE patients. Serum IL-2 level was measured using the ELISA method, while Treg and Th17 counts were measured using flow cytometry technique. The Systemic Lupus Activity Measure (SLAM) score was used to assessed SLE disease activity because it is the easiest and most efficient, does not require a high cost, reliable, valid, responsive to changes in disease activity over time, with sensitivity and specificity equivalent to SLEDAI. To avoid bias in the results of the study, the study subjects were naive SLE patients who had never received prior intervention. This study aimed to regarding the relationship between Treg/Th17 and various cytokines with disease activity in SLE patients at Dr. Soetomo Hospital, Surabaya.

METHODS

This was an observational cross-sectional analysis study. The study was conducted at dr. Soetomo General Hospital, Surabaya. The population studied were all patients with SLE who were treated as an inpatient at dr. Soetomo General Hospital, Surabaya. A consecutive sampling method was used to select the participant for this study.

Criteria for inclusion in this study were that the subjects agreed to participate in the study, patient with SLE based on ACR criteria, active SLE based on SLAM score, and aged 16-60 years. Patients that were or have been receiving steroid and or immunosuppressant therapies, patients with infection, tuberculosis, morbus hansen, HIV/AIDS, malignancy, asthma, acute coronary syndrome, mixed connective tissue disease and smoking were excluded.

Serum IL-2 level was measured using ELISA, while Treg and Th17 counts were measured using flow cytometry. SLAM scores was used the assessed SLE disease activity.

Data were analyzed with analytic statistics and presented in tables, graphics and diagrams. Correlation between serum IL-2 level and Treg/Th17 ratio was analyzed using Spearman's correlation test. Statistical analysis was performed using the SPSS software version 23.0, and a p value <0.05 was considered statistically significant. The Ethics Committee Health Research of Faculty of Medicine, Universitas Airlangga has approved this study with 0346/KEPK/VI/2018.

RESULTS

Thirty SLE patients who were treated at the inpatient department of the internal medicine department of the dr. Soetomo General Hospital, Surabaya, Indonesia who met the inclusion and exclusion criteria were enrolled in this study, consisting of thirty (100%) females. General characteristics of study subjects are shown in Table 1. Data such as age, hemoglobin, WBC, lymphocyte, ESR, C3 and SLAM score were normally distributed, whereas data such as thrombocyte, CRP and C4 were not normally distributed. Test of normality were performed on all data using Shapiro-Wilk test.

The diagnosis of SLE in this study was assessed using the ACR 1997 criteria, fulfilling at least 4 of 11 symptoms and signs. Table 2 shows the distribution of clinical and laboratory manifestations of research subjects based on ACR 1997 criteria.

Serum IL-2 levels, Treg percentage, Th17 percentage and Treg/Th17 ratio were measured on all study samples. The normality test using Saphiro-Wilk showed abnormal distribution of IL-2, Treg percentage and Treg/Th17 ratio, whereas Th17 percentage showed normal distribution. The results of serum IL-2 levels, Treg percentage, Th17 percentage and Treg/Th17 ratio are shown in Table 3. For comparison, measurements were made on healthy subjects with the same methods and examination tools. In healthy subjects the median of serum IL-2 level was 49.31 pg/mL; the median of Treg percentage was 11.75%; the average of Th17 percentage was 13.01 ± 0.91%; and the Treg/Th17 ratio was 0.830. Figure 1 shows there was a significant correlation (r_s = 0.463; P = 0.010) between IL-2 and Treg/Th17 ratio.

Table 1: General characteristics of the patients

Indicator	Results (n=30)				
	Frequency (%)	Mean±SD	Median	Min	Max
Age		31 ±10			
16-20 year	4 (13%)				
20-30 year	12 (40%)				
30-40 year	8 (27%)				
>40 year	6 (20%)				
Hemoglobin (g/dL)		7.1±2.3			
< 12.0	29 (96.7%)				
> 12.0	1 (3.3%)				
WBC (cell/mm^3)		8056±3442			
< 4000	1 (3.3%)				
> 4000	29 (96.7%)				
Lymphocyte (cell/mm^3)		968±428			
< 1500	27 (90%)				
> 1500	3 (10%)				
Thrombocyte (cell/mm^3)			190500	2000	744000
< 100000	12 (40%)				
> 100000	18 (60%)				
ESR (mm/hour)		62±38			
CRP (mg/dL)			7.84	0.10	290.77
C3 (mg/dL)		28.88 ±12.98			
C4 (mg/dL)			15.25	5.00	54.30
SLAM		29 ±4			

Table 2: Distribution of clinical and laboratory manifestations based on ACR 1997 criteria

ACR 1997 criteria	Total	Percentage (%)
Malar rash	4	13.3
Discoid rash	8	26.7
Photosensitivity	13	43.3
Oral Ulcer	10	33.3
Arthritis	23	76.7
Serositis	14	46.7
Kidney Disorder	8	26.7
Neurological Disorders	6	20.0
Hematological Disorders		
Hemolytic Anemia	4	13.3
Leukopenia < 4000/mm^3	1	3.3
Lymphopenia < 1500/mm^3	27	90.0
Thrombocytopenia < 100.000/mm^3	12	40.0
Immunology Disorder:		
Anti ds-DNA negative (≤ 92.6 WHO unit/mL)	18	60
Anti ds-DNA positive (> 92.6 WHO unit/mL)	12	40
ANA *test*:		
Negative (< 20 unit)	7	23.3
Positive (≥ 20 unit)	22	73.3

Table 3: Serum IL-2 levels, Treg and Th17 percentage, Treg/Th17 ratio

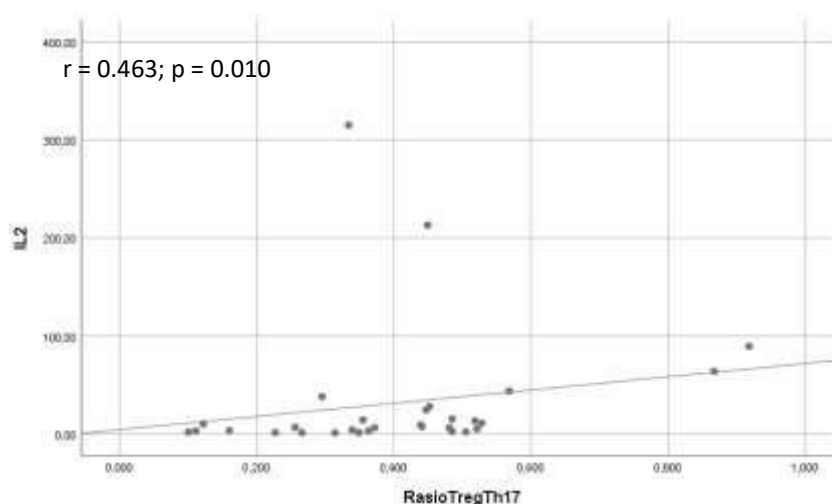| Indicator	Results (n=30)		
	Results	Minimum	Maximum
Treg (%)	11.92	1.39	41.20
Th17 (%)	30.09 ± 12.60	5.62	56.90
Treg/Th17 ratio	0.405	0.100	0.918

Fig. 1: Correlation between serum IL-2 level and Treg/Th17 ratio in active SLE using Spearman test, r showed the correlation coefficient.

DISCUSSION

There was a significant correlation between serum IL-2 levels and Treg/Th17 ratio in active SLE. The decline in development of IL-2 affects the balance of Treg/Th17 and induces a transition to T17. In SLE, there is a disturbance of Treg and Th17 balance in the form of a shift towards Th17. Furthermore, there is a decrease in the Treg/Th17 ratio. The moderate size of correlation coefficient might be due to other cytokines that play a role in Treg/Th17 balance such as TGF-β, TNF-α, IL-1β, IL-6, IL-10, IL-21, and IL-23 [2,9].

From the total of thirty patients involved in this study, all patients (100%) were female. Other studies also reported female dominance in SLE [20–22]. These studies support the theory that SLE affects women more than men, presumably due to the influence of the hormones estrogen and prolactin. However, another report showed case in male who had six signs and symptoms, such as malar rash, arthritis, photosensitivity, anemia, immunologic disorder, and antinuclear antibody [23].

The mean age of the study subjects were almost the same when compared to some previous studies [16,20–22]. These studies support the theory that SLE is more common in reproductive age, presumably due to the influence of the hormones estrogen and prolactin.

The most common clinical manifestations based on the ACR 1997 criteria were arthritis, obtained in 76.7% of the study subjects. Several other studies also reported arthritis as the most clinical manifestation [20,24,25]. Arthritis can be found as an initial manifestation at the time of diagnosis or as an accompanying manifestation of flare. Some flares are caused by bacterial infections [26,27]. These studies support the theory that musculoskeletal manifestations are prominent and often found in active SLE.

This study used the SLAM score to assess disease activity and obtained an average SLAM score of 29.3. The score <20 indicates the disease is inactive, while the score ≥ indicates the disease is active. In this study, all study subjects had SLAM scores >20 so it was concluded that all subjects in this study were in active disease condition. All subjects in this study were naive patients who had not received corticosteroid therapy or other immunosuppressants. Various studies reported that Asian races are associated with higher disease activity [28–30]. A study in Yogyakarta found that the average SLAM score of hospitalized SLE patients was by 16.7 [30]. Whereas, research conducted in 3 countries with a predominantly white population found that the average SLAM score were lower at 4.1 in America, 6.3 in the UK, and 7.3 in Canada [31]. These studies support the theory that SLE disease activity in Asian populations is higher than that of white populations. The study by reported a lower SLAM score than this study, it might be related to the effect of therapy.

Indeed, Treg can affect individual cells' ability to generate cytokines of effectors [32]. For Treg and Th17 cell growth, IL-2 is a cytokine that is essential. The differentiation of Treg effector cells is influenced by IL-2, so in low IL-2 conditions there is a decrease in the amount of Treg. IL-2 plays a role in the expansion and conversion of CD4 + Foxp3 T cells into CD4 + Foxp3 + or Treg T cells. In the pathogenesis of SLE, besides affecting Treg, IL-2 also affects Th17. IL-2 is known to inhibit IL-17A expression and differentiation of Th17 cells. IL-2 inhibits differentiation of Th17 through activation of STAT5, decreased RORγt, and production of Ets-1 [33–35].

Some limitation of this study that could influence the results, among others, are that this study was a cross-sectional research so that the serum IL-2 level, Treg and Th17 percentage, Treg/Th17 ratio measurement was only done once. Moreover, serum IL-2 levels, Treg and Th17 percentages, Treg/Th17 ratio do not have a universal reference value that applies universally.

CONCLUSION

There was a significant correlation between serum IL-2 levels and Treg/Th17 ratio in active SLE . In active SLE, there is a decrease in IL-2 levels which does not fully affect the Treg/Th17 balance. It is thought that there are other cytokines that affect the balance. Further research need to be done with a comparative study between healthy and SLE naive patients with a larger sample size and balanced proportions between the two groups. A pre- and post-research is needed to assess changes in IL-2 levels, Treg percentage, Th17 percentage, Treg/Th17 ratio, and SLE disease activity after administration of steroid therapy and immunosuppressants. Various other cytokines and chemokines that might be involved in the Treg/Th17 balance in SLE disease need to be studied and then analyzed for their strength and effects with each other.

CONFLICT OF INTEREST

The author stated that there is no conflict of interest.

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