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Interleukin-34 and Disease Activity in Systemic Lupus Erythematosus Patients

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ABSTRACT

Systemic Lupus Erythematosus (SLE) is characterized by exacerbation and remission, which needs close monitoring of the disease activity. Systemic lupus erythematosus disease activity can be determined by the SLE Disease Activity Index (SLEDAI) score. Evaluation of the disease activity is essential to be a guidance for treatment. Interleukin-34 (IL-34) is related to the pathogenesis of SLE. Serum IL-34 can be a candidate marker to evaluate SLE disease activity, and it is correlated with the SLEDAI score. This study aimed to determine the correlation between IL-34 level and disease activity in SLE patients based on the SLEDAI (Mex-SLEDAI) score. An observational analytical study with a cross-sectional design was carried out in six months (June-November 2019) in 27 SLE patients in the Department of Internal Medicine, Faculty of Medicine, Sumatera Utara University/Adam Malik General Hospital, Medan. Systemic lupus erythematosus disease activity was measured based on the Mex-SLEDAI score. Serum and urine were collected to obtain the Mex-SLEDAI score and IL-34 level. IL-34 level was measured in all subjects by using Enzyme-Linked Immunosorbent Assay (ELISA). Spearman correlation test was used to determine the correlation between IL-34 level and disease activity in SLE patients based on the SLEDAI (Mex-SLEDAI) score. There was a significant correlation between IL-34 level and disease activity in SLE patients based on SLEDAI (Mex-SLEDAI) score ($r=0.965$, $p < 0.001$). Further studies were needed with a sample of SLE patients in a balanced proportion based on their disease activity to obtain representative IL-34 levels in SLE patients based on their disease activity.

Keywords: Systemic lupus erythematosus, interleukin-34, SLEDAI, Mex-SLEDAI

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic systemic autoimmune disease marked by dysregulated autoantibody production and complement activation leading to multiple organ damage, especially in kidneys, blood system, and central nervous system.[1]

Interleukin (IL)-34 is a novel cytokine that does not have significant amino acid sequence homology with other cytokines. IL-34 is expressed in the spleen, thymus, heart, brain, lung, liver, kidney, testis, prostate, ovary, small intestine, and colon. Interleukin-34 is another ligand of the Colony-Stimulating Factor-1 receptor (CSF-1R). Interleukin-34 binds to CSF-1R and stimulates the differentiation and proliferation of lymphocytes and cytokines expression, resulting in inflammatory lesions and autoimmunity. IL-34 can promote the expression of IL-6, interferon γ-Inducible Protein (IP) 10, and Monocyte Chemoattractant Protein-1 (MCP-1) in the whole human blood. Interleukin-6, IP10, and MCP-1 are involved in the pathogenesis of SLE. [1–3]

Incidence and prevalence data of SLE vary among countries. Currently, WHO reports five million lupus patients worldwide. Most of them are females of childbearing age, and every year more than one hundred thousand new patients are found. In Indonesia, the exact number of lupus is unknown. The number of lupus cases in 2016 was almost twice that in 2014. The prevalence of SLE in the community based on Kalim *et al.* in Malang was 0.5% of the total population.[4]

The diagnosis of SLE requires clinical and laboratory criteria. Based on the American College of Rheumatology (ACR) Classification criteria revised in 1997, diagnostic criteria of SLE consists of 11 items on a list, include malar rash, discoid rash, photosensitivity, oral ulcer, arthritis, serositis, renal disorder, neurological, hematological, and immunological disorder and positive Anti-Nuclear Antibody (ANA). The diagnosis of SLE is made if 4 of 11 criteria are found.[5]

Systemic lupus erythematosus is characterized by exacerbation and remission, which needs close monitoring of the disease activity. Systemic lupus

erythematosus disease activity can be determined based on the SLE Disease Activity Index (SLEDAI) score. Evaluation of the disease activity is essential to be a guidance for treatment.[6]

The interleukin-34 level significantly correlated with the SLE disease activity based on the SLEDAI (Mex-SLEDAI) score. This statement was in line with Xie *et al*., which showed a positive correlation between IL-34 levels and the SLEDAI score (r=0.319, p=0.004). Similar results were also found in the study of Wang *et al.* in which a significant positive correlation between IL-34 level and the SLEDAI score was found. Interleukin-34 can be used as a marker of SLE disease activity. There has been no study about this in Indonesia, especially in Medan. Therefore, this study aimed to determine the correlation between IL-34 level and disease activity in SLE patients based on the SLEDAI (Mex-SLEDAI) score.[1,7]

METHODS

This study was an observational analytical study with a cross-sectional design performed on SLE patients in the Department of Internal Medicine, Faculty of Medicine, Sumatera Utara University/Adam Malik General Hospital, Medan. Subjects were recruited from June to November 2019 consecutively. The inclusion criteria were patients diagnosed with SLE according to American College of Rheumatology (ACR) classification criteria revised in 1997, patients without SLE medication for a week before enrollment of the study, aged > 18 years old and patients who have signed informed consent. The exclusion criteria were SLE patients with malignancy, immunocompromised condition, infection, diabetes, and obesity. Ethical clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Sumatera Utara University/Adam Malik General Hospital, Medan with number 612/TGL/KEPK FK USU-RSUP HAM/2019.

Medical history and physical examination results of each subject were collected. Serum samples were taken from all subjects upon admission. The serum was stored at an optimal temperature and kept freeze until the test. Serum IL-34 level was measured using human IL-34 ELISA kit by Bioassay Technology Laboratory with sandwich ELISA principle. Urine samples were also collected for urinalysis. Systemic lupus erythematosus disease activity was measured by using the MEX-SLEDAI score. Patients with a score of less than two were reported as remission or inactive lupus, 2–5 were reported to have a probability of flare, and scores more than five were reported as lupus flare or active lupus.[8,9]

Data were analyzed using computer statistic software. Categorical variables were shown in frequency (n) and percentage (%). Numerical variables were shown in mean and Standard Deviation (SD) for normally distributed data, but median for data without normal distribution. Data normality was analyzed using the Sapphiro-Wilk test ($n \leq 50$) and, p-value < 0.05 was stated statistically significant. Spearman correlation test was used to determine the correlation between IL-34 level and disease activity in SLE patients based on the Mex-SLEDAI score. Kruskal-Wallis test was used to determine the comparison of IL-34 level based on disease activity in SLE patients.

RESULT AND DISCUSSION

A total of 27 female SLE patients were enrolled in this study.

Table 1. Baseline characteristics of research subjects

Variable	N	Mean±SD Median (min-max)
Age (years)	27	28.19±8.33
ANA test (IU/mL)	27	108,0 (75.3-246.7)
Anti ds-DNA (IU/mL)	27	464,0 (202,0-716,3)
Hb (g/dL)	27	8.93±2.48
Reticulocyte (%)	27	1.63 (0.25-16.42)
White blood cell count (/µL)	27	4680 (1410-18820)
Platelet count (/µL)	27	197000 (9000-441000)
Creatinine (mg/dL)	27	4.18 (0.64-6.84)
IL-34 (ng/L)	27	168,26 (33.10-384,76)
Mex-SLEDAI score	27	11.48±7.40

Systemic lupus erythematosus is more frequent in females, with a female-to-male ratio of 9:1. Hormones (primarily estrogens), possible risk genes for SLE in the X chromosome (which are pivotal in determining sex hormone levels), and some immunologically relevant genes (interferon-related and CD40 ligand) probably explain the prevalence of lupus among females. Estrogens may enhance SLE by prolonging autoimmune cells' survival, increasing T-helper type 2 (Th2) cytokine production, and stimulating B-cells to produce autoantibodies. The inhibition of the Th1 response and the enhancement of CD40L expression on lupus T-cells may indirectly promote the Th2 response and lead to further B-cell hyperactivity.[10,11]

Measurement of SLE disease activity based on the Mex-SLEDAI score showed that most subjects in this study (74.1%) were diagnosed with lupus flare or active lupus, 18.5% possibility of flare, and 7.4% with remission or inactive lupus. Flare was defined generally based on one or more of the following parameters: increase in disease activity score assessed by a validated index; the appearance of new or worsening of disease manifestations, (e.g., increase in proteinuria in the case of renal flares); sufficient treatment intensification (e.g., an increase of steroid dosage). Risk factors for flare in patients with SLE include poor compliance with treatment, low C3/C4, high anti-ds-DNA, and quick tapering or withdrawal of maintenance of immunosuppressive therapy. In this study, most subjects had poor compliance with treatment, leading to an increased risk of flare.[12]

Spearman correlation test showed a significant correlation between IL-34 level and SLEDAI (Mex-SLEDAI) score (r=0.965; p < 0.001), suggesting a powerful and positive correlation between IL-34 and Mex-SLEDAI score. This result was in line with Xie *et al*., which showed a positive correlation between IL-34 levels and SLEDAI score (r=0.319, p=0.004). The study of Wang *et al*. showed IL-34 levels were positively correlated SLEDAI score (r=0.62; p=0.0011). IL-34 can be used as a marker of disease activity.[1,7]

Systemic lupus erythematosus is a chronic systemic autoimmune disease characterized by dysregulated autoantibody production and complement activation, leading to multiple organ damage, especially in kidneys, blood system, and central nervous system.[1]

Interleukin-34 shares a common receptor with Macrophage-Colony Stimulating Factor (M-CSF). As another ligand of the Colony-Stimulating Factor-1 Receptor (CSF-1R), IL-34 binds to CSF-1R and stimulates the differentiation and proliferation of lymphocytes and expression cytokines, resulting in inflammatory lesions and autoimmunity. Interleukin-34 can promote the expression of IL-6, interferon γ-IP 10, and MCP-1 in the whole human blood. Interleukin-6, IP 10, and MCP-1 are involved in the pathogenesis of SLE.[1]

There is growing evidence that IL-34 influences different immune cells and is associated with immune and inflammatory responses, therefore affecting the onset and progression of various diseases. In patients with lupus flare with peak level of the immune and inflammatory responses, IL-34 circulates in higher levels to mediate immune tolerance and reduce inflammatory lesions. Notably, the growing evidence suggests a correlation between IL-34 and disease severity, chronicity, and progression. Through a complex signaling network that requires various types of cells and molecules, IL-34 plays a critical role in orchestrating innate and

Table 2. Disease activity in SLE patients based on Mex-SLEDAI score

SLE Disease Activity	N	%
Lupus flare or active lupus (score > 5)	20	74.1
Probability of flare (score 2–5)	5	18.5
Remission or inactive lupus (score < 2)	2	7.4
Total	**27**	**100**

Table 3. Correlation between IL-34 Level and Mex-SLEDAI score

Variable	SLEDAI (Mex-SLEDAI) Score	
	r	p
IL-34 (ng/L)	0.965	< 0.001

*) Spearman correlation test

adaptive immune responses during inflammation. The expression of IL-34 can be developed by various stimuli related to inflammation, such as proinflammatory cytokines, PAMPs, infections, chemical stressors, and tissue injuries. Multiple studies have revealed that IL-34 expression is regulated by NF-kB, a central molecule that governs all signaling pathways relevant to inflammation.[13,14]

Kruskal-Wallis test showed a significant difference from the mean of IL-34 levels based on SLE patients' disease activity ($p < 0.001$). IL-34 level was significantly higher in subjects with lupus flare or active lupus (248.82 ng/L) than those who had a probability of flare (47.30 ng/L). In comparison, the remission or inactive lupus subjects (33.43 ng/L) found the lowest level. These results were in line with the study of Wang *et al*., which showed a significant difference in IL-34 levels in active SLE patients (median 312 pg/mL) compared with inactive SLE patients (median 97 pg/mL; $p < 0.001$).[7]

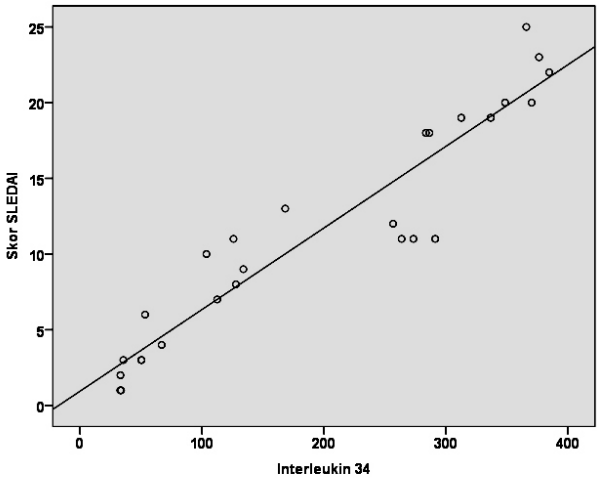

Figure 1. Scatterplot graph of the correlation between IL-34 level and Mex-SLEDAI

There is growing evidence that IL-34 influences different immune cells and is associated with immune and inflammatory responses, therefore affecting the onset and progression of the disease. In patients with lupus flare, the peak level of immune and inflammatory responses leads to higher IL-34 levels in the circulation to mediate immune tolerance and reduce inflammatory lesions that occur.[15]

There was a significant correlation between IL-34 and the five components of the Mex-SLEDAI score, i.e. neurological disorders, renal disorders, arthritis, mucocutaneous disorders, and serositis. According to the Spearman correlation test, IL-34 showed a strong correlation with neurological disorders and renal disorders ($p < 0.001$) with correlation values (r) of 0.797, and 0.792, respectively. Besides, IL-34 showed a moderate correlation with arthritis, mucocutaneous disorders, and serositis with

Table 4. Comparison of IL-34 level according to the disease activity in SLE patients based on Mex-SLEDAI score

Disease Activity in SLE Patients	IL – 34 Level (ng/L) Mean±SD	p
Lupus flare or active lupus (score > 5)	248,82 (107,02)	< 0.001
Probability of flare (score 2 - 5)	47.30 (13.61)	
Remission or inactive lupus (score < 2)	33.43 (0.46)	

*) Kruskal-Wallis test

Table 5. Correlation between IL-34 level and the Mex-SLEDAI score components

Variable	IL-34 Level	
	r	p
Neurological disorders	0.797	< 0.001
Renal disorder	0.792	< 0.001
Vasculitis	NA	NA
Hemolysis/thrombocytopenia	0.343	0.08
Miositis	NA	NA
Arthritis	0.483	0.011
Mucocutaneous disorder	0.498	0.008
Serositis	0.418	0.03
Fever/fatigue	0.254	0.200
Leucopenia/lymphopenia	0.355	0.07

*) Spearman correlation test

correlation values (r) of 0.483, 0.398, and 0.418, respectively.

Interleukin-34 has essential roles in maintaining CNS homeostasis by reacting to several cell types comprising neurons, microglia, and endothelial cells. CNS capillary endothelial cells also express CSF1R and respond to IL-34 by upregulating various tight junction molecules, suggesting an essential role for IL-34 in maintaining the Blood-Brain Barrier (BBB) integrity. Interleukin-34 secretion is increased in damaged neurons, mediating neuronal rescuing by autocrine activation of survival pathways in injured neurons, and mediating paracrine effects on microglia, stimulating microglial proliferation and neuroprotective functions denoted by phagocytosis of toxicants and damaging debris, and produces anti-oxidant enzymes.[13]

Practically, IL-34 promotes the local proliferation of macrophages that mediate TEC destruction. After being released into circulation, IL-34 stimulates the proliferation of myeloid cells in the bone marrow, thus enriches the circulation with a large pool of myeloid cells, consequently recruited by IL-34-induced chemokines at the inflamed kidney.[13] Evidently, IL-34 also acts as a negative regulator of skin inflammation.[13]

CONCLUSION AND SUGGESTION

A significant difference from IL-34 levels was found based on the disease activity in SLE patients. There was a significant correlation between IL-34 level and disease activity in SLE patients based on the SLEDAI (Mex-SLEDAI) score. Interleukin-34 also significantly correlated with the five components of the Mex-SLEDAI score, i.e. neurological disorders, renal disorders, arthritis, mucocutaneous disorders, and serositis. Further studies were needed with a sample of SLE patients in a balanced proportion based on their disease activity to obtain representative IL-34 levels in SLE patients based on their disease activity.

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