

Correlation between serum leptin concentration and disease activity in normal body mass index premenopausal women with systemic lupus erythematosus

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ABSTRACT

Background. Leptin is recognized as a cytokine-like hormone with pleiotropic actions in modulating immune responses. The role of leptin in pathogenesis of systemic lupus erythematosus (SLE) was not fully understood yet. Previous study did not find the correlation between serum leptin concentration and disease activity in patients with SLE, but selection of the subjects wasn't based on the classification of body mass index (BMI) and menopausal status.

Objective. To determine correlation between serum leptin concentration and the disease activity in normal BMI premenopausal women with SLE.

Methods. Serum leptin concentration was measured by enzyme-linked immunosorbent assay and disease activity was scored using Mexican SLE disease activity index (Mex-SLEDAI). Spearman's correlation coefficient test was used for evaluating the strength of association between leptin level and Mex-SLEDAI score.

Results. Seventy normal BMI premenopausal women with SLE were included in this study. The median of serum leptin concentration was 13.4 (0.6 – 45.9) ng/ml. The median serum leptin concentration in patients with active disease was 12.4 (0.6 – 41.6) ng/dl, whereas in patients with inactive disease was 15.2 (3.9 – 45.9) ng/dl. No significant different was found between serum leptin concentration in active and inactive disease ($p = 0.14$). A weak negative correlation was observed between leptin concentration and Mex-SLEDAI score, but not statistically significant ($r = -0.22$; $p = 0.07$).

Conclusion. No correlation was found between serum leptin concentration and disease activity in normal BMI premenopausal women with SLE. Prednisone doses and disease duration might interfere.

cell survival and stimulating the production of pro-inflammatory cytokines such as interferon- γ and interleukin (IL)-2. Recent evidence demonstrates a detrimental involvement of leptin in promoting the pathogenesis of various autoimmune diseases. In respect of its diverse functions in immunity, leptin has been explored as a potential target for therapeutic development in treating autoimmune diseases.⁵

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by widespread inflammation affecting virtually every organ or system in the body. The disease is associated with the deposition of auto antibodies and immune complexes, leading to tissue damage.⁶ Role of leptin in pathogenesis of SLE is not fully understand yet. A cross-sectional study conducted by Garcia-Gonzales et al found that patients with SLE had higher leptin serum leptin concentration than control group.¹ A borderline negative correlation was observed between leptin concentration and disease activity (Mexican SLE disease activity index [Mex-SLEDAI] score), but not statistically significant. Selection of the subjects in their study wasn't based on the classification of body mass index (BMI) and menopausal status, so the study result might be interfered by those variables. The aim of this cross-sectional study is to determine correlation between serum leptin concentration and disease activity in SLE premenopausal women with normal BMI.

MATERIALS AND METHODS

Characteristic of systemic lupus erythematosus patients

Seventy SLE premenopausal women with normal BMI according to Asia-Pacific criteria were included in the study, all of whom gave written consent.⁷ The SLE patients were diagnosed according to the revised American College of Rheumatology criteria.^{8,9} Subjects were collected from outpatient unit Department of Internal Medicine, Cipto Mangunkusumo Hospital and Yayasan Lupus Indonesia in Jakarta. The approval of the Local Ethical Committee to carry out the research had been obtained before.

Leptin constitutes a hormone synthesized by the adipose tissue that binds with a receptor which is a member of the class I cytokine receptor family.¹⁻

⁴ Leptin has been increasingly recognized as a cytokine-like hormone with pleiotropic actions in modulating immune responses. Leptin can activate monocytes, dendritic cells and macrophages then stimulate them to produce Th1 type cytokines. Leptin also exerts activating effects on neutrophils and natural killer cells and stimulate their gene expressions. Importantly, leptin has been shown to modulate the adaptive immunity by enhancing T

Patients with diabetes mellitus, hypertension, chronic renal failure, hyperthyroidism, malignancy, pregnancy, oral contraceptive therapy, severe infection at the time of assessment, history of surgery within a month before initiation of the study or smoking habit were excluded. Included in the assessment were clinical variables such as age, body mass index, disease duration, mean of daily prednisone doses in the previous month and disease activity according to the Mex-SLEDAI.¹⁰ Patients with a Mex-SLEDAI score greater than or equal to 2 points were considered active while inactive disease if the score was less than 2 points.¹¹

Serum leptin concentration evaluation

Serum leptin concentration was measured by enzyme-linked immunosorbent assay (Quantikine, R&D systems, Inc., Abingdon, USA). Blood samples were taken after at least 12 hours overnight fasting. The serum was frozen at -20°C until the analysis of leptin concentration. All procedures for the measurement of leptin were made by the same researcher.

Statistical analysis

Statistical analysis were done using SPSS version 13. Considering that leptin concentration was abnormally distributed, we used the medians and ranges for statistical comparisons. Spearman's correlation coefficient test was used for evaluating the strength of association between leptin concentration and disease activity. The Mann-Whitney U-test was used for comparisons between the leptin concentration in patients with active and inactive disease. Statistical significance was set at the < 0.05 level.

RESULTS

Serum leptin concentration in SLE patients range from 0.6 to 45.9 ng/ml with median of 13.4 ng/ml. The median disease duration was two years (range : 0.2 to 18 years), the median daily doses of prednisone was 10 mg (range : 0 to 55 mg) and the median Mex-SLEDAI score was 2 (range : 0 to 12). These variables distribution was shown in table 1.

Table 1 Characteristic distribution of systemic lupus erythematosus patients

Characteristics	Median (range)*
Age (years)	26 (16 – 46)
BMI (kg/m^2)	21.1 (18.6 – 22.9)
Disease duration (years)	2 (0.2 – 18)
Prednisone doses (mg/day)	10 (0 – 55)
Mex-SLEDAI score	2 (0 – 12)
Serum leptin concentration (ng/ml)	13.4 (0.6 – 45.9)

* Data are abnormally distributed

Of all the subjects, 40 (57%) patients had active disease (Mex-SLEDAI score ≥ 2 points) and 30 (43%) patients had inactive disease (Mex-SLEDAI < 2 points). Patients with active disease had lower of serum leptin concentration than patients with inactive disease, but the difference was not statistically significant, as shown in table 2 ($p = 0.14$).

Table 2 Serum leptin concentration based on disease activity

Disease activity	n	Median (range)	p value*
Active	40	12.4 (0.6 – 41.6)	0.14
Inactive	30	15.2 (3.9 – 45.9)	

*Comparison made with the Mann-Whitney U test

Serum leptin concentration or score Mex-SLEDAI between SLE patients who only got prednisone therapy and combination therapy (prednisone and others immunosuppressant) was not significantly different, as shown in table 3 ($p = 0.49$ and $p = 0.79$, respectively).

Table 3 Serum leptin concentration and Mex-SLEDAI score based on kind of therapy

Parameter	Kind of therapy		P value*
	Prednisone only	Combination	
	Median (range)	Median (range)	
Serum leptin concentration	12.35 (0.70-45.86)	16.39 (0.62-42.39)	0.49
Mex-SLEDAI score	2 (0-12)	2 (0-10)	0.79

*Comparisons made with the Mann-Whitney U test

Using Spearman's correlation test, a weak negative correlation was observed between serum leptin concentration and Mex-SLEDAI score, but was not statistically significant, as shown in figure 1 ($r = -0.22$, $p = 0.07$).

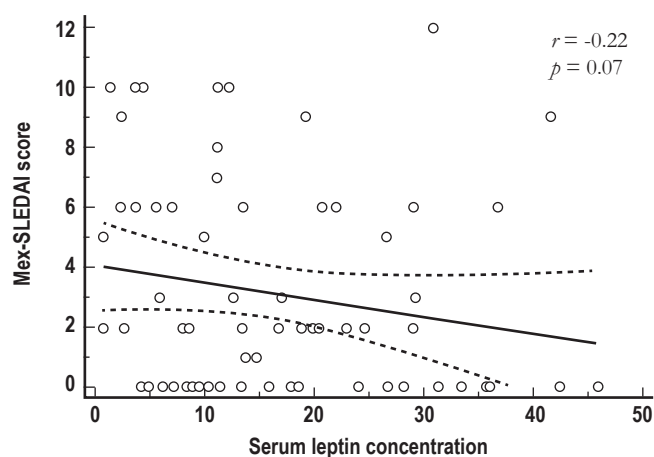


Figure 1 Correlation between serum leptin concentration and Mex-SLEDAI score in normal body mass index premenopausal women with systemic lupus erythematosus

Systemic lupus erythematosus patients with renal disorder or vasculitis had lower serum leptin concentration than patients without this symptom, whereas SLE patients with arthritis or mucocutaneous disorder had higher serum leptin concentration than patients without this symptom. However, all of this different were not statistically significant (table 4).

Table 4 Serum leptin concentration based on the clinical picture

Symptoms	Serum leptin concentration [Median (range)]		p value*
	Symptom present	Symptom absent	
Arthritis (n = 6)	17.15 (2.53 – 29.2)	13.39 (0.62 – 45.86)	0.97
Mucocutaneous disorder (n = 16)	19.48 (0.62 – 41.62)	11.75 (0.7 – 45.86)	0.06
Hematologic disorder (n = 5)	11 (0.62 – 19.1)	13.53 (0.7 – 45.86)	0.31
Renal disorder (n = 20)	11.03 (1.25 – 41.62)	16.23 (0.62 – 45.86)	0.09
Vasculitis (n = 9)	4.19 (1.25 – 30.8)	14.56 (0.62 – 45.86)	0.05

*Comparisons made with the Mann-Whitney U test

Analysis of confounding variables

Prednisone doses and disease duration might take place as confounding variables. By using Spearman's correlation coefficient test showed that the main variables (serum leptin concentration and Mex-SLEDAI score) correlated with confounding variables (table 5).

Table 5 Correlation between main and confounding variables

Main variables	Confounding variables			
	Prednisone doses		Disease duration	
	correlation coefficient (r)	p value	correlation coefficient (r)	p value
Serum leptin concentration	-0.1	0.41	0.326	0.006
Mex-SLEDAI score	0.396	0.001	-0.028	0.816

DISCUSSION

The median serum leptin concentration in normal BMI premenopausal woman with SLE was 13,4 (range : 0.6 – 45.9) ng/ml in this study. This result was lower than the result of Garcia-Gonzales et al study, which was 30 (range : 4 – 74) ng/ml, while Wislowska et al found the median serum leptin concentration was 7.5 (range : 1.8 – 66.3) ng/ml.^{1, 12} Both of these studies did not do subject selection based on BMI and menopausal status. Therefore these different results might be affected by those variables because of their effect on leptin concentration. Both of these studies showed median concentration of serum leptin in normal woman was 15 (range: 2 – 59) ng/ml and 8.8 (range: 0.7 – 39.2) ng/ml. Several factors known to affect leptin concentration are gender, body weight, BMI and corticosteroid. This study only considered female subject to control the effect of gender on leptin concentration.

Glucocorticoid increased leptin production *in vitro* and a hexogen glucocorticoid on human increased leptin concentration in the circulation.^{13,14} However, Tataranni et al found that either acute or prolonged administration of glucocorticoid (methylprednisolone) did not affect plasma leptin concentration on human.¹⁵ Bokarewa et al reported that administration of disease-modifying antirheumatic drugs (DMARDs) such as methotrexate (MTX), sulfasalazine, azathioprine, cyclosporine, or tumor necrosis factor (TNF)- α

inhibitor on rheumatoid arthritis patient affected plasma leptin concentration. They reported that subjects with DMARDs therapy beside MTX had low leptin concentration compared to subject who got MTX therapy. On the other hand, Harle et al reported that there was no change of leptin serum concentration on rheumatoid arthritis patient who got adalimumab (anti-TNF- α) therapy.¹⁷ Teplan et al reported that subject who had kidney transplantation with long term mycophenolate mofetil therapy had a decrease plasma leptin concentration.¹⁸ In this study, we did not find any difference on serum leptin concentration between subject received prednisone therapy only and subject received combination therapy of prednisone and another immunosuppressant. This result showed administration of another immunosuppressant combined with prednisone did not affect serum leptin concentration.

A patient was considered to have an active disease activity if his/her Mex-SLEDAI score ≥ 2 points, and inactive disease activity if his/her Mex-SLEDAI score < 2 points.¹⁹ In this study, patients with active disease activity had median serum leptin concentration lower than patients with inactive disease activity. However this difference was not statistically significant. There was no correlation found between serum leptin concentration and Mex-SLEDAI score in patients with active disease activity and patients with inactive disease activity (data wasn't shown).

In our study, a negative correlation was found between serum leptin concentration and disease activity (Mex-SLEDAI score). However, this correlation was not statistically significant. This result was similar with previous studies done by Gracia-Gonzales et al and Wislowska et al.^{1,12} Both of these studies resulted in a negative correlation between serum leptin concentration and the disease activity measured with Mex-SLEDAI respectively without statistic significance. In this study, this result might be caused by : (1) Mex-SLEDAI score is a clinical instrument for disease activity, which does not reflect changes on cytokines or hormones concentration that influence the inflammatory response; (2) Effects of confounding variables (prednisone doses and disease duration); and (3) Lack of the study's power (this study's power was 80%).

Systemic lupus erythematosus is characterized by a predominantly humoral response (Th2 type) that leads to over expression of Th2 cytokines, such as IL-4 and IL-10.²⁰ Th2 immune responses leads to B cell hyperactivity and production of pathogenic auto antibodies, then finally, tissue injury and damage.^{6,21,22} Leptin exerts inhibitory effects on Th2 cells which in turn decreases production of IL-4 and IL-10 and then reduces activation of B cells.⁵ This process causes decrease in disease activity.^{5,23} This might explained negative correlation between serum leptin concentration and disease activity found in our study.

Significant negative correlation between serum leptin concentration and disease activity was found by Kumpers et al on the ANCA-associated Vasculitis study. In this study, disease activity was measured with CRP concentration, Birmingham Vasculitis Activity Score, and Circulating Endothelial Cells.²⁴

Experimental study conducted by Sarraf et al found that serum leptin concentration promptly increased during

acute infection and sepsis.²⁵ It was consistent with raised leptin mRNA expression during acute inflammation reaction stimulated by LPS and cytokines like TNF- α , IL-6 and IL-1 β . This result showed that these cytokines stimulated leptin release into circulation following acute inflammation. On the other hand, Popa et al study on rheumatoid arthritis (RA) found negative correlation between serum leptin concentration and markers of inflammation such as CRP and IL-6.²⁶ The conclusion of their study was a significant inverse correlation between inflammation and leptin concentrations in active RA patients.²⁶ This suggested that active chronic inflammation may decrease plasma leptin concentrations.

Bokarewa's study on RA showed that leptin concentration in synovial fluid RA patients with erosive arthritis was lower than RA patients without erosive arthritis.²⁷ They concluded that local consumption of leptin in the joint cavity was associated with non-erosive joint disease, suggesting that leptin has a protective role against the destructive course of RA.

A cohort study done by van der Helm-van Mil et al showed that RA patients (anti-CCP positive) with high BMI (≥ 30 kg/m²) had lower disease activity than RA patients with BMI less than 30 kg/m² during 3 years observation.²⁸ They concluded a positive correlation between BMI and serum leptin concentration. Necla et al found a negative correlation between serum leptin concentration and presence of proteinuria in nephritic syndrome patients.²⁹ Until now, there has not been any prospective study conducted to determine leptin role on prognosis of SLE patients. Therefore, further investigation is needed to evaluate whether leptin has protective or destructive effect on SLE patients.

In this study, serum leptin concentration in SLE patients who had clinical picture of renal disorder, arthritis, vasculitis,

muco-cutaneous and hematologic disorder didn't differ with SLE patients without those clinical picture. In contrary, Wislowska et al showed significantly lower serum leptin concentration in SLE patients with clinical picture of arthritis or neurologic disorder than patients without this symptom.¹²

The main variables (serum leptin concentration and Mex-SLEDAI score) had a correlation with confounding variables (prednisone doses and disease duration) after being analyzed with statistic means. Serum leptin concentration had a positive correlation with disease duration, whereas Mex-SLEDAI had a positive correlation with prednisone doses. This suggested that our study results might be interfered with confounding variables.

Our study had some limitations, including wide range of prednisone doses and disease duration made subjects became less homogeneous. There were also common limitations of cross-sectional study such as an unavoidable presence of the incidence-prevalence bias. It was difficult for a cross-sectional study design to determine relation between leptin concentrations with clinical manifestations of SLE. Stronger designs such as cohorts and clinical trials allowed investigators to obtain better conclusions about effects of leptin on clinical manifestations in SLE patients.

In conclusion, the result of this study showed no correlation between serum leptin concentration and disease activity in SLE premenopausal women with normal body mass index. Several confounding variables such as prednisone doses and disease duration might interfere to these results. Further investigations using stronger design are necessary to show the relation between serum leptin concentration and cytokines produced by Th1 and Th2 cells. It is better to conduct the study in new patients with normal BMI who haven't received steroid therapy to minimize the confounding variables

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