Comparison of leptin serum levels between systemic lupus erythematosus (SLE) and non-SLE patients at Mohammad Hoesin Hospital Palembang

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Abstract. Systemic lupus erythematosus (SLE) is an autoimmune disease, in which immune system mistakenly attacks healthy tissues such as joint, skin, kidneys, brain, and other organs. The dysregulation occurs in the immune system and produces various inflammatory autoantibodies and cytokines in SLE patients. Leptin plays a role in the immune system's reaction to autoimmune diseases related to the inflammatory response, therefore it acts as a potential target in the development of therapy in the autoimmune disease. Serum leptin levels in SLE patients may be elevated, and are associated with the involvement of lupus nephritis, although it still unclear. A cross sectional study was conducted with a number of 70 subjects, each group consisted 35 SLE and 35 non-SLE patients who were treated as outpatient in the period of April - November 2016 at internal medicine Mohammad Hoesin hospital Palembang. We found that, leptin serum levels in the SLE group were higher than the non-SLE group but showed no significantly different.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease that usually affects many organ systems with many clinical manifestations, progression of disease and prognosis. This disease mainly affects women in reproductive age with a high mortality rate. Genetic, immunologic, hormonal and environmental factors are thought to play roles in its pathophysiology [1,2].

In SLE, dysregulation occurs in cell T or B immune system and the non-steroid immune system in which tolerance and production of various inflammatory autoantibodies and cytokines are impaired [3–5]. The incidence of lupus has tripled in the last forty years, especially in mild case findings, due to the developing of diagnostic tools nowadays. In addition, with the various treatment modalities that have grown rapidly today, patients have a higher life expectancy, eventhough in lupus patients also have high comorbidities [6,7].

There is an interaction between genetic, environmental and hormonal factors that produce an abnormal immune response as an underlying pathogenesis. Disorder of the immune system regulatory mechanism in SLE, characterized by immune system irregularities involving B cells, T cells and monocyte cells that cause polyclonal cell B activation, increase the production of autoantibodies and
immune complex formation. Excessive and uncontrolled T cells will help differentiate and activate autoantibodies formed by B cells.

Activation of T cells and B cells is caused by the presence of specific external antigen stimulations such as chemicals, bacterial DNA, viral antigens, cell wall phospholipids or self-derived (self antigen) DNA and RNA proteins then it will stimulate the formation of antibodies [3,8,9]. Antigens will be carried by Antigen Presenting Cell (APC) or bind to antibodies on the surface of B cells, then carried to T cells via MHC molecules on the surface and interact with TCR. The cytokines produced by T cells will affect and stimulate B cells, transferring the antibody class from IgM to IgG that has a high affinity for binding to ds-DNA.

The immune system disorders in SLE can be as immune complex clearance disorders, immune complexes implantation in the liver, and decreased up-take of immune complexes in the spleen. It is also associated with the deficiency of complement components C1, C2, C4. The presence of the disorder leads to increased exposure of antigen to the immune system and the occurrence of immune complex deposition in various organs resulting in complement fixation in the organ. This event leads to complement activation resulting in inflammatory mediators that induce an inflammatory reaction which eventually leads to tissue destruction [3,9–11].

Leptin is a cytokine-like hormone, a non-glycosylated peptide of 16 kDa molecular weight, in humans comprising 167 amino acid sequences. Leptin is produced primarily in adipocyte cells of white adipose tissue and is produced by the Ob / Lep gene located on chromosome 7 in humans. Leptin is also produced with lower levels by other tissues, such as the stomach, musculoskeletal, placenta and bone marrow [12,13].

Leptin plays a role in the immune system's reaction to autoimmune diseases related to the inflammatory response, so leptin is a potential target in the development of therapy in the autoimmune disease. Serum leptin levels in lupus patients may be elevated, and are associated with the involvement of lupus nephritis, although not correlated with disease activity [14]. Similarly, Gonzalez et al's studies have shown an increase serum leptin levels in SLE patients [15].

Therefore, a study is needed to determine the serum leptin level in SLE patients and compare it with non-SLE patients in the Internal Medicine Polyclinic at Mohammad Hoesin Hospital, Palembang.

2. Methods
This study aimed to determine serum leptin levels in patients with SLE and non-SLE at Mohammad Hoesin Hospital, Palembang. This research was an observational study with cross sectional design, conducted in the internal medicine outpatient of Mohammad Hoesin Hospital, Palembang Indonesia from April to December 2016 and the biomolecular laboratory of the Medical Faculty, Universitas Sriwijaya. Sample size are 70, each group consist 35 SLE and 35 non-SLE patient. The inclusion criteria are patients who were treated in the period of April - November 2016 and signed informed consent. Exclusion criteria are other autoimmune disease, malignancy, severe infection and obesity. Non-SLE group is a healthy people or only mildly ill and has fulfilled the inclusion and exclusion criteria. Method of leptin examination using the ELISA method and the Reader 680 tool with leptin reagent R & D Systems (DL00).

3. Results
Baseline characteristics of sample can be seen in the table 1 involving gender, age, level of education, occupation, and BMI. In this study the sex of all research samples were women, each group consists of 35 subjects. The results of unpaired T-Test analysis comparing BMI between SLE (20,58±2,32 kg/m²) and non-SLE (21,25±2,01 kg/m²) group showed non significantly different (p=0,202).

SLE patients who became the study samples had many clinical complaints related to arthritis and mucocutaneous compared with neurological clinical complaints, nephropathy, vasculitis, haemolysis, miosis, serositis, fever and fatigue. and leukopenia and lymphopenia. The duration of SLE pain in the study subjects was almost over 3 months or chronic, and so was the duration of treatment. Research subjects obtained MEX SLEDAI Score mostly had moderate-severe SLE disease activity, only 1 subject had mild disease activity.
Table 1. Baseline characteristics of subjects.

| Characteristic | Category        | Group      | Total n(%) |
|----------------|----------------|------------|------------|
|                | Non-SLE n(%)     | SLE n(%)   |            |
| Age            | 12 - 25 year     | 18 (25.7)  | 8 (11.4)   | 26 (37.1) |
|                | 26 - 45 year     | 14 (20)    | 25 (35.7)  | 39 (55.7) |
|                | 46 - 65 year     | 3 (4.3)    | 2 (2.9)    | 5 (7.2)   |
| Level of Education | Primary school | 0 (0)      | 6 (8.6)    | 6 (8.6)   |
|                | Junior High School | 0 (0)    | 3 (4.3)    | 3 (4.3)   |
|                | Senior High School | 2 (2.9)   | 15 (21.4)  | 17 (24.3) |
|                | Diploma          | 14 (20)    | 4 (5.7)    | 18 (25.7) |
|                | Bachelor         | 18 (25.7)  | 6 (8.6)    | 24 (34.3) |
|                | Master           | 1 (1.4)    | 1 (1.4)    | 2 (2.9)   |
| Occupation     | Unemployed       | 2 (2.8)    | 24 (34.3)  | 26 (37.1) |
|                | Employed         | 33 (47.1)  | 11 (15.7)  | 44 (62.9) |
| BMI            | Underweight      | 3 (4.3)    | 10 (14.3)  |           |
|                | Normal           | 24 (34.3)  | 21 (30)    | 45 (64.3) |
|                | Overweight       | 8 (11.4)   | 7 (10)     | 15 (21.4) |

Table 2. Clinical characteristics of SLE patients.

| Characteristics | Category | Total (%) |
|-----------------|----------|-----------|
| Neurology symptom | Yes      | 3 (8.6)   |
|                  | No       | 32 (91.4) |
| Nephropathy      | Yes      | 10 (28.6)|
|                  | No       | 25 (71.4)|
| Vasculitis       | Yes      | 5 (14.3) |
|                  | No       | 30 (85.7)|
| Hemolysis        | Yes      | 6 (17.1) |
|                  | No       | 29 (82.9)|
| Miosistis        | Yes      | 0 (0)     |
|                  | No       | 35 (100) |
| Arthritis        | Yes      | 22 (62.9)|
|                  | No       | 13 (37.1)|
| Mucokutaneus     | Yes      | 2 (5.7)   |
|                  | No       | 33 (94.3)|
| Serositis        | Yes      | 2 (5.7)   |
|                  | No       | 33 (94.3)|
| Fever            | Yes      | 7 (20)    |
|                  | No       | 28 (80)  |
| Leukopenia / Lymphocytopenia | Yes | 10 (28.6)|
|                  | No       | 25 (71.4)|
| Mex SLEDAI Score | Mild     | 1 (2.9)   |
|                  | Moderate | 18 (51.4)|
|                  | Severe   | 16 (45.7)|
| Duration of SLE  | < 3 Month | 7 (20)    |
|                  | 3 – 12 Month | 14 (40)|
|                  | > 12 Month  | 14 (40)  |
| Duration of SLE Treatment | < 3 Month | 8 (22.9)|
|                  | 3 – 12 Month | 14 (40)|
|                  | > 12 Month  | 13 (36.1)|

Clinical characteristics of SLE patients based on MEX SLEDAI score components such as table 2. Laboratory characteristics of research subjects obtained according to table 3. Subjects had a mean Hemoglobin level of 12.1 g/dL which showed sufficient Hb levels, although Hb levels in the SLE group were significance lower than in the non-SLE group because anemia is common in SLE patient.
The number of leukocytes and platelets showed normal mean values, both in the SLE and non-SLE groups are not significantly different, whereas the ESR levels showed a slight increase in all study subjects, especially significantly in the SLE group, this may be due to inflammatory activity in SLE patients.

**Table 3. Characteristics of laboratory subjects.**

| Characteristics       | Mean (±SD) | Total Mean (±SD) | P     |
|-----------------------|------------|------------------|-------|
| Hemoglobin (gr/dL)    | 13,03 (±0,92) | 11,17 (±1,91) | 12,1 (±1,76) | 0,000* |
| WBC (/mm³)            | 7,397,14 (±1899,41) | 8,234,60 (±4422,60) | 7,815,8 (±3378,33) | 0,798* |
| Platelet (/μL)        | 283,914,29 (±93,889,29) | 283,685,71 (±106,140,24) | 283,800 (±99,473,48) | 0,716* |
| ESR (mm/hours)        | 23,49 (±17,09) | 34,11 (±19,19) | 28,8 (±18,81) | 0,005* |

*a*Mann Whitney Test  
*b*Unpaired T-Test

This study also analyzed the correlation between serum leptin levels and MEX SLEDAI score using spearmen test, with the results that shows no correlation.

**Table 4. Correlation between leptin serum levels with MEX SLEDAI Score.**

| Characteristic     | Mean (±SD) | n   | p    | r    |
|-------------------|------------|-----|------|------|
| Leptin (pg/ml)    | 292,09 (±416,34) | 35  | 0,465| -0,128* |
| MEX SLEDAI Score  | 7,14 (±5,95)   |     |      |      |

*a*Spearman test

Leptin levels in the SLE group had higher mean leptin levels than the non-SLE group, as shown in Table 5. Statistical test results using Mann-Whitney test showed no significant differences in serum leptin levels in the SLE and non-SLE groups.

**Table 5. Comparison of leptin levels in groups of SLE and non-SLE**

| Group   | Mean (±SD) pg/ml | p    |
|---------|------------------|------|
| SLE     | 292,09 (±416,34) | 0,725* |
| Non-SLE | 220,40 (±159,05) |      |

*a*Mann-Whitney Test

4. Discussions

SLE disease moreover often occurs in productive age women than man, with a high mortality rate. The annual incidence of SLE in the United States is 5.1 per 100,000 population, while the prevalence of SLE in the United States is 52 cases per 100,000 population, with the ratio of women and men between 9:14 : 1. The age group that suffers from SLE are mostly found in the middle age, and began to decline in the elderly [2]. In this study results were obtained according to middle age to 45 years. The education level of subjects in this study is mostly high, as well as in the SLE group. Two-thirds of subjects in this study are still working, while in the SLE group are only one-third are still working, this may be due to the illness that inhibit working activities. The BMI was within normal range, but in the SLE group it was more lower than non-SLE group, that could be caused by the illness.

Immune system consist innate and acquired in which leptin plays an important roles. It’s immunological roles related to its molecular structure that has high affinity to long-chain helical cytokine family such as IL-2 or IL-12. Leptin activate monocytes or macrophages to produce proinflammatory cytokines (TNF-α, IL-6), to induce phagocytosis and expression of type 2 cyclooxygenase (COX2). Leptin promotes chemotaxis and activation of neutrophils and natural killer cells (NK). Leptin is involved in lymphocytes functions. The adipokine induces activation and proliferation.
of naïve T cells, by production of IL-2. The immune responses of T helper 1 (Th1) are promoted by leptin, whilst the function and proliferation of T regulatory cells are inhibited [16].

In this study, inflammatory activity occurred in the SLE group. This can be seen from the significant increase of ESR serum levels compared to the non-SLE group. There were no lymphocyte count data to compare in the two groups, although the incidence of leukopenia or lymphocytopenia about 40% occurred in the SLE group. ESR data that shows the inflammatory process in the SLE group are supported by the MEX SLEDAI score, which is moderate to severe SLE disease activity.

Leptin levels in SLE patients are still unclear. Li et al concluded that the results of meta-analysis of various studies related to serum leptin levels in SLE patients did not show significant differences compared with healthy subjects [17], although this was different from the results of meta-analysis performed by Olazagasti et al and Lee et al [18,19].

All above study has support by Mohammed et al that levels of serum leptin between active and inactive SLE patient was not significant statistical differences. Likewise, correlations between serum leptin levels and disease activity measured by SLEDAI was not significant different [20].

Factors which may influence the results of this study are most of subjects in SLE groups had suffered the illness for more than 3 months and / or had received immunosuppressant therapy (steroids and mofetil mycophenolate). Although most of the SLE disease activity was moderate to severe, the results of the analysis showed no correlation between serum leptin levels and MEX SLEDAI scores in determining disease activity.

However, from the results of this research, it must be admitted that many samples are needed to analyze the comparison of serum leptin levels in patients with SLE and non-SLE to be sufficient and get strong conclusions. Therefore, these findings can be further investigated by different methods and larger sample sizes.

5. Conclusions
Leptin serum levels in the SLE patient higher than the non-SLE patient, but has no significant.

6. References
[1] Schmeding A and Schneider M 2013 Fatigue, health-related quality of life and other patient-reported outcomes in systemic lupus erythematosus Best Pr. Res Clin Rheumatol. ;. doi 10.1016/j.berh.2013.07.009. 27 363–75
[2] Kasjmir Y, Kalim H and Wijaya L 2011 Rekomendasi perhimpunan rheumatologi Indonesia untuk diagnosis dan pengelolaan lupus eritematosus sistemik (Jakarta)
[3] Tutuncu Z N and Kalunian K C 2013 The definition and classification of systemic lupus erythematosus Dubois’ lupus erythematosus ed D J Wallace and B H Hahn (Philadelphia: Williams & Wilkins) pp 16–20
[4] Hahn B H 2015 Systemic Lupus Erythematosus Harrison’s Principles of Internal Medicine ed D L Longo, A S Fauci, D L Kasper, S L Hauser, J L Jameson and J Loscalzo. (New York: McGraw-Hill Medical) pp 1432–7
[5] Petri M 2005 Review of classification criteria for systemic lupus erythematosus Rheumatic Disease Clinics of North America ed M Urowitz (Saunders) pp 245–54
[6] Ajeganova S, Gustafson T, Jogestand T, Froastagard J and Hafstrom I 2015 Bone mineral density and carotid atherosclerosis in systemic lupus erythematosus: a controlled cross-sectional study Arthritis Res. Ther. 17
[7] Yamada A and Salama D 2002 The role of novel T cell costimulatory pathways in autoimmunity and transplantation J Am Soc Nephrol. 13 559–75
[8] Hassan S, Rana M, Leveille C, Nadiri A, Jundi M, Polyak M and El-Fakhry, Youssef Mourad W M 2009 Implication of CD154/CD40 interaction in healthy and autoimmune responses Curr. Immunol. Rev. 15 285–99
[9] Rahman A and Isenberg A 2008 Mechanism of disease systemic lupus erythematosus N.Engl.J.Med 358 929–39
[10] Wofsy D and Daikh D 2002 Treatment of autoimmune diseases by inhibition of T cell
costimulation *Mod Rheumatol* **12** 1–4

[11] Horwitz A and Gray D 2007 The interaction of T cells with cells of the innate immune system and B cells in the pathogenesis of SLE *Dubois’ Lupus Erythematosus* ed D Wallace and B Hahn pp 132–52

[12] Lago F, Dieguez C, Reino J and Gualillo O 2007 Adipokines as emerging mediators of immuneresponse and inflammation *Nat. Clin. Pract. Rheumatol.* **3** 716–24

[13] Gomez R, Lago F, Reino J, Dieguez C and Gualillo O 2009 Adipokines in the skeleton: influence on cartilage function and joint degenerative diseases *J. Mol. Endocrinol.* **43** 11–8

[14] Barbosa V, Francescantonio P and Silva N 2015 Leptin and adiponectin in patients with systemic lupus erythematosus: clinical and laboratory correlations *Rev Bras Reum.* **55** 140–5

[15] Gonzalez A, Lopez L, Gonzalez I, Munoz E, Paramo M, Abundis E and Nava J 2002 Serum leptin levels in women with systemic lupus erythematosus *Rheumatol. Int.* **22** 138–41

[16] Margiotta D, Vadacca M, Navarini L, Basta F and Afeltra A 2006 The Complex Role of Leptin in SLE: Is Leptin A Key Link between Metabolic Syndrome, Accelerated Atherosclerosis and Autoimmunity? *Lupus Open Access* **1**

[17] Li H, Zhang T, Leng R, Li X, Li X and Pan H 2015 Plasma/serum leptin levels in patients with systemic lupus erythematosus: a meta-analysis *Off. J. Inst. Mex. del Seguro Soc.* **46** 551–6

[18] Olazagasti J, Wetter D, Chowdhary V and Reed A 2014 Adipokine levels in adult patients with systemic lupus erythematosus: a meta-analysis *Mayo Found. Med. Educ. Res.*

[19] Lee Y H and Song G G 2018 Association between circulating leptin levels and systemic lupus erythematosus: an updated meta-analysis *Lupus* **27**

[20] Mohammed S F, Abdalla M, Ali, Ismaeil W M and Sheta M M 2018 Serum leptin in systemic lupus erythematosus patients: Its correlation with disease activity and some disease parameters *Egypt. Rheumatol.* **40** 23–7

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