Serum IL-17 levels correlate with urinary albumin in systemic lupus erythematosus (SLE) pregnant mice model

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ABSTRACT

Women of reproductive age are more likely to have systemic lupus erythematosus (SLE), which frequently results in health issues, particularly during pregnancy. A normal pregnancy's first trimester shows a marked increase in the percentage of Th17 cells, which then steadily declined in the second and third trimesters. Meanwhile, IL-17 level increases in SLE-affected pregnant women. This study aimed to analyze the correlation between serum IL-17 and pregnancy outcome (fetus weight, blood pressure, urinary albumin) in SLE pregnant animal models. Twenty mice were randomly divided into two groups, including the normal pregnant group and SLE-pregnant group. The SLE pregnant mice was made by intraperitoneally induction of 0.5 mL pristane. Serum IL-17 was assayed by enzyme-linked immunosorbent assay (ELISA) method. The serum IL-17 level, the blood pressure and urinary albumin were significantly higher in the SLE pregnant mice group than those of the normal pregnant group (p< 0.05). The weight of fetus was significantly smaller in the group of SLE pregnant mice group than the normal pregnant group (p< 0.05). There was a significantly positive correlation between the serum IL-17 level and urinary albumin (p = 0.042; r = 0.459). In conclusion, serum IL-17 levels correlate with urine albumin in SLE pregnant models, but do not correlate with fetus weight and blood pressure.

ABSTRAK

Wanita usia subur cenderung mengalami systemic lupus erythematosus (SLE), yang seringkali menimbulkan masalah kesehatan, terutama selama kehamilan. Prosentase sel Th17 meningkat pada trimester pertama kehamilan normal, yang kemudian menurun pada trimester kedua dan ketiga. Sementara itu, kadar IL-17 meningkat pada wanita hamil dengan SLE. Penelitian ini bertujuan untuk mengkaji hubungan antara serum IL-17 dan luaran kehamilan (berat janin, tekanan darah, albumin urin) pada model SLE bunting. Dua puluh mencit secara acak dibagi menjadi dua kelompok, termasuk kelompok bunting dan kelompok bunting dengan SLE. Model SLE pada mencit dibuat dengan induksi 0,5 mL pristan secara intraperitonial. Kadar IL-17 serum ditetapkan dengan enzyme-linked immunosorbent assay (ELISA). Kadar IL-17 serum, rerata tekanan darah dan albumin urin lebih tinggi secara nyata pada kelompok tikus SLE bunting dibandingkan kelompok bunting normal (p<0,05). Berat janin lebih kecil secara nyata pada kelompok tikus SLE bunting dibandingkan dengan kelompok bunting normal (p<0,05). Terdapat hubungan positif yang signifikan antara kadar IL-17 serum dan albumin urin pada model SLE bunting, tetapi tidak berhubungan dengan berat janin dan tekanan darah.

Keywords:
serum IL-17; systemic lupus erythematosus; pregnancy outcome; urine albumin; animal model

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multisystemic involvement. It affects practically any organ system and can result in irreversible damage. The etiology of SLE remains incompletely understood and it is believed to be a combination of genetic, hormonal, environmental, and immune factors. This autoimmune disease has a significant female predominance. The onset during reproductive years, coupled with improved survival, has led to increased numbers of pregnancies in women with SLE. The pregnancy outcomes have also significantly improved. The rate of pregnancy loss has decreased from 43% to 17% in recent years. However, SLE patients have smaller children than normal individuals, and SLE pregnancy still carries a high risk of complications. The autoreactive adaptive arm (T and B lymphocytes) represents a prominent role in SLE physiopathology, leading to the subsequent production of antinuclear autoantibodies and the consequent deposition of immune complexes throughout the body that can directly induce inflammation. This sustained response causes patients to develop local inflammatory episodes that give rise to a vicious circle in the autoimmune response, leading to a number of immunological abnormalities and tissue destruction. In addition, the involvement of CD4+ T helper cells (Th) in SLE has become increasingly evident and disturbances in the expression of the Th1/Th2/Th17 cytokine network have also been reported in SLE patients.

SLE is more common in women of reproductive age and often causes health problems especially during pregnancy. Pregnant women suffering from SLE have bad complications for the mother and fetus. During pregnancy, when remission SLE before pregnancy less than 6 months, the risk of exacerbation of SLE is 50%. In the first trimester of a normal pregnancy, the percentage of Th17 cells were significantly increased and then gradually decreased in the second and third trimesters. Other studies mention that normal pregnant women had lower number of Th17 cells. Meanwhile, pregnant women with SLE contained elevated levels of IL-17. Nonetheless, the pathomechanism of pregnancy in SLE, especially the relationship between the output SLE pregnancies remains unclear. Therefore, this study aimed to analyze the correlation between serum IL-17 with pregnancy outcome in SLE pregnancies models.

MATERIAL AND METHODS

Animals

Female BALB/c mice, 26-28 wk of age, weighing 25–30 g, were used for this study. Twenty mice were randomly divided into two groups with ten mice in each group. The first group was a normal pregnant group as control group. The second group was a SLE-pregnant group (SLE group). Mice were housed in a clean wire cage and maintained under standard laboratory conditions with temperature of 25 ± 3°C and dark/light cycle 12/12 h. Standard diet and water were provided ad libitum. Mice were acclimatized to laboratory conditions for one wk before the experiment. Mice care and experimental procedures were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

Pristane-induced SLE

SLE model was made according to Satoh et al. by an intraperitoneally injection of 0.5 mL pristane (Santa Cruz Biotechnology, USA) at 6-8 wk of age and after 7 d of acclimatization. This SLE model was reported to reproduce...
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clinical similarities to human SLE and being useful to identify and validate new therapies for the treatment or prevention of this autoimmune disease. After 12 wk of pristane induction, clinical and immunological manifestations experienced by BALB/c mice were observed. The clinical manifestation included hair loss, facial redness, ascites, and intestinal dilatation. While, the immunological manifestation was an increase in antinuclear antibody (ANA). Followed, after 12 wk of pristane induction, mice fertilization was performed.

Breeding

Breeding began after 12 wk of pristane induction. Mice were caged in a constant photoperiod. Virgin female mice were mated with fertile male of the same strain to induce pregnancy. The following morning of finding a vaginal plug was designated as gestational day (GD) 1.18

Tissue and blood sampling

Blood samples were taken at day 18 of pregnancy. Surgery and blood collection were carried out at the Pharmacology Laboratory of Universitas Brawijaya, Malang, Indonesia. The sampling stage was started with preparing tools and materials for minor operations such as tweezers, scissors, ether and vacutainers without EDTA. Then the mice were euthanized by anesthetizing with 10% chloroform formalin inhalation to perform surgery to obtain blood. Blood was taken from the heart at approximately 1 mL using a syringe, and then the blood that was drawn was put into a vacutainer tube without anticoagulant and then centrifuged to get serum at 3500 rpm for 15 min. After being centrifuged, the supernatant was taken and transferred to an Eppendorf tube and then stored at -40°C.

Urinary sampling

Urinary albumin measurement was performed once on the 14th day of gestation. Urine samples were obtained the day before the height of urine albumin levels. All the mice were placed in special cages to collect urine for 24 h. Then in the morning, the mice's urine was collected, taken, and stored in a labeled urine bottle. The urine was then transferred into an Eppendorf tube and centrifuged at 3500 rpm for 15 min, and then the urine sample is stored at -20°C.

Analysis of serum IL-17

Analysis of serum IL-17 was performed using IL-17 ELISA kit (Biolegend, USA, Catalog No: 432507). The analysis was conducted according to the detailed instruction in the kit. After blood was taken and put into a blood collection tube without EDTA, blood was centrifuged to obtain supernatant at 35000 rpm for 15 min. Then IL-17 levels were measured by measuring IL-17 cytokine secretion in serum using the ELISA Kit mouse IL-17A method. Overall, the procedure for measuring IL-17A levels was carried out based on standard methods from the Biolegend factory (Biolegend, USA, Catalog Number 432507). Measurement of IL-17A levels began with washing buffer dilution (20x). Aquades was added to 1 mL of wash buffer concentrate, then 2 mL of aquabidest was added to the matrix. One mL of assay buffer was added to standard IL-17 mice. The final standard concentration after dilution was 1000 pg/mL. Standards were prepared using 8 Eppendorf tubes, then standard wells and sample wells were prepared and then washed using wash buffer 4 times.

Fifty µL of each matrix were added to the standard wells, and 50 µL of serum samples were put to the sample wells. The wells were then rotated to ensure that the solution was homogeneous. Then,
the wells were incubated at 37°C for 120 min. After incubation, the wells were washed four times with wash buffer, and 100 µL mouse IL-17A detection antibody was added to all wells (standard and sample). Then the wells were rotated so that the solution was homogeneous. The wells were incubated again at 37°C for 60 min, and then the wells were washed four times with a wash buffer. 100 µL of HRP A was added to each well, and then the wells were rotated so that the solution was homogeneous, then incubated again at 37°C for 30 min. The wells were washed four times with wash buffer. After washing, 100 µL of substrate solution was added to each well. The color changed to blue (in the darkroom). Incubation were added for another 30 min, then 100 µL stop solution was added to the well. the color changed to yellow. The results were read on the Elisa Reader Microplate machine at \( \lambda \) 450 nm.

Measurement of mean arterial blood pressure

Blood pressure was measured using a Kent Scientific CODA. Blood pressure measurement was performed by calculating a mean arterial blood pressure (MAP) of the pregnant SLE group and then compared to the average of the normal pregnant group.

Analysis of urinary albumin

Analysis of urinary albumin was performed using ELISA kit (Elabscience, Catalog No; E-EL-M0656). According to the kit’s comprehensive instructions, the analysis was conducted on 12 h urine samples. The centrifuged urine sample was stored at −20°C and removed from the refrigerator, waiting for it to thaw. The required urine sample was 100 µL. The ELISA kit was removed from the refrigerator 30 min before use, and standard wells and samples were prepared. Wash buffer (25x) was prepared by adding 24 mL of distilled water with 1 mL of concentrated wash buffer. The standard was prepared, and 1 mL of diluent and reference samples were added and then allowed to stand for 10 min (after the standard was diluted, it would be 1000 pg/mL). The standard was made using 8 Eppendorf tubes, and then 100 µL standard was put into the well and a 100 µL urine sample was added. After that, the well containing the standard solution and the urine was rotated for 5 min to homogenize the solution.

The wells were incubated at 37 °C for 90 min. Then the wells were washed with wash buffer 5x. Each well received 50 µL of Biotin Ab solution, which was subsequently homogenized by rotating the wells. The process was followed with incubation at 37°C for 60 min. The next stage was incubated at 37°C for 60 min. After incubation, the wells were washed with wash buffer 3x. Fifty µL HRP conjugate was added to each well. Then the wells were rotated so that the solution was homogeneous. The wells were incubated again at 37°C for 30 min. Then the wells were washed five times with wash buffer. After washing, 90 µL of substrate solution was added to each well, and the color changed to blue (in the darkroom). Incubate for 15 min, then stop solution is added to the well, and the color changes to yellow. The results are read on the ELISA reader machine. Urinary albumin levels with a unit value of µg/mL were recorded and analyzed from the results obtained.

Measurement of fetal weight

Fetal weight was measured using analytical scales METTLER AE50.
Ethics

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine Brawijaya University.

Statistical analysis

All variables met the parametric prerequisite test. All data proved to be customarily distributed then data analysis was continued with a correlation test to determine the relationship between IL-17 serum levels and pregnancy outcomes (urine albumin levels, blood pressure, and fetal weight) using the Pearson correlation test.

RESULTS

The clinical manifestations in pristane-induced SLE mice model

Clinical manifestations in pristane-induced SLE mice model are presented in FIGURE 1. Among the ten pristane-induce SLE mice model, 5 (50%) mice experienced ruffled feathers, 2 (20%) mice had facial flushing, 1 (10%) mice had ascites (10%), 1 (10%) mice had gut dilatation and 1 (10%) mice had no clinical manifestations of SLE (TABLE 1).

FIGURE 1. Clinical manifestation of SLE mice, including ruffled feathers (A), facial flushing (B), ascites (C), and gut dilatation (D).
The IL-17 serum levels

Serum IL-17 levels were significantly higher (p=0.042) in the SLE pregnant mice group (1076 ± 11.4 ng/dL) than the normal pregnant mice group (1006 ± 101.3 ng/dL) (FIGURE 2).

The outcome of pregnancy

Pregnancy outcome of normal pregnant mice and SLE pregnant mice group including the arterial pressure, urinary albumin, and fetal weight are presented in TABLE 2. The blood pressure of the SLE pregnant mice group (91.8 ± 22.32 mmHg) were significantly higher than those the normal pregnant group (69.8 ± 7.62 mmHg) (p = 0.013). Urine albumin levels of the SLE pregnant mice group (1402.3 ± 401.5 ng/mL) were also significantly higher than those the normal pregnant group (132.3 ± 197.9 ng/mL) (p= 0.000). Whereas, fetal body weight of the SLE pregnant mice group (0.8 ± 0.2 g) were significantly lower than those the normal pregnant group (1.1 ± 0.1 g) (p= 0.000).

The correlation of biomarkers with pregnancy outcome

TABLE 3 presents the correlation of serum IL-17 with pregnancy outcome. The levels of serum IL-17 was significantly correlated with urinary albumin levels (p = 0.042; r = 0.459). There was no significant correlation between serum IL-17 with mean blood pressure and fetal body weight.

TABLE 1. The distribution of clinical manifestations in pristane-induced SLE mice model

<table>
<thead>
<tr>
<th>The clinical manifestations</th>
<th>Pristane-induced SLE mice [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruffled feathers</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Facial flushing</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Ascites</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Gut dilatation</td>
<td>1 (10)</td>
</tr>
<tr>
<td>No clinical manifestation</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>
Serum IL-17 levels correlate...

FIGURE 2. Serum IL-17 level in the normal pregnant and the SLE pregnant mice groups (*significant different p<0.05.)

TABLE 2. The outcome of pregnancy in each group

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Normal pregnant (n=10)</th>
<th>SLE pregnant (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg)</td>
<td>69.80 ± 7.62</td>
<td>91.8 ± 22.32</td>
<td>0.013</td>
</tr>
<tr>
<td>Urine albumin level (ng/mL)</td>
<td>132.3 ± 197.9</td>
<td>1402.3 ± 401.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Fetal body weight (g)</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.004</td>
</tr>
</tbody>
</table>

TABLE 3. The correlation between biomarkers and pregnancy outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Urinary albumin</th>
<th>Blood pressure</th>
<th>Fetal body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17</td>
<td>p=0.042*; r=0.459</td>
<td>p=0.172; r=0.318</td>
<td>p=0.894; r=-0.032</td>
</tr>
</tbody>
</table>

DISCUSSION

Systemic lupus erythematosus (SLE) is a potentially severe autoimmune disease characterized by increased titers of serum autoantibodies. Common autoantibody-mediated damage mechanisms in SLE include immune complex-mediated damage, cell surface binding, and cytotoxicity, reactivity with autoantigens expressed on apoptotic or activated cell surface, penetration into living cells, binding to cross reactive extracellular molecules. In this study, the levels of serum IL-17 of the SLE pregnant mice group were significantly higher than those the normal pregnant group (p < 0.05). A previous study reported that injection of pristane could overstimulate immune
response reaching auto reactive level. SLE is associated with impaired disposal of apoptotic cell products due to deficiencies of scavenging molecules in phagocytic cells, such as scavenger receptors, or complement components such as C1q which facilitate phagocytosis of apoptotic cells. These apoptotic cells can induce and augment Th17 and the doubly potent Th1/Th17 responses. In patients with SLE, there is an increased percentage of Th17 cells and its activity which is accompanied by the rising of the cytokine IL-17. In this study, there is a significant positive correlation between the levels of serum IL-17 with urinary albumin (p = 0.042; r = 0.459). This finding indicated that serum IL-17 might be correlated with the changes in glomerular barrier alteration. Our finding was in line with previous studies that IL-17 might be correlated with the level of disease activity on the SLE. An increase in the activity of Th17 cells can induce the production of inflammatory mediators and toxic to the tissue.

**CONCLUSION**

In conclusion, there is a correlation between serum IL-17 levels with urinary albumin in pregnant SLE mice models. However, there is no correlation between serum IL-17 levels with fetus weight and blood pressure.

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