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Relationship of TNF-α levels in the Placenta with Blood Pressure, Urinary Albumin, and Fetal Weight Balb/c Strain Mice in Pregnant SLE Models

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Abstract

Systematic Lupus Erythematosus (SLE) is one of the autoimmune diseases which, if it occurs at reproductive age, can cause problems during Pregnancy. Tumor Necrosis Factor Alpha (TNF-α) is a proinflammatory cytokine that develops follicles during ovulation, corpus luteum formation, and endometrial function. Therefore, if the Pregnancy increases, it will affect the output results. This study aims to determine the relationship between TNF-α placenta levels with blood pressure, urinary albumin, and fetal body weight in mice of the BALB/c strain of the lupus bunting model. This study was a true experimental (actual experimental) conducted in vivo using balb/c mice experimental animals. The treatment group was given an intraperitoneal injection of 0.5 ml pristane.TNF-α levels were measured using the ELISA method, blood pressure mice with a tail-cuff computerized system tool, and weighing fetal body weight with analytical scales.

The result of this study was that there was a significant difference between TNF-α levels between the control and treatment groups. There were significant differences in pregnancy outcomes in the form of blood pressure and urinary albumin in the control and treatment groups. TNF- α levels were associated with increased urine albumin ($p=0.01<\alpha$). It can be concluded that there is a relationship between the levels of TNF- α in the placenta to the increase in urinary albumin.

Keywords: SLE; TNF- α placenta level; Balb/c Mice; Pregnancy Outcome

1. Introduction

Systematic lupus erythematosus (SLE) is one of the autoimmune diseases. The disease is chronic and multi-systemic, caused by the deposition of immune complexes with diverse clinical manifestations of several body organs. The cause of SLE is not yet clear but is suspected to be related to several factors such as genetic, environmental, and hormonal that cause immune response abnormalities[1].

Overall, this disease affects more women of reproductive age, with a ratio of women versus men is 9:19:1. [2] Pregnant women with SLE can increase the risk of complications and worsen their pregnancy condition, and complications can occur in the mother and fetus related to immune mechanisms. Complications that can occur in pregnant women with SLE include preeclampsia, prematurity, abortion, impaired fetal growth, and fetal death [3,4].

In SLE patients, there are obstacles to eliminating the accumulation of immune complexes in the circulation, so that deposition in the tissues impacts tissue damage due to the reactive production of intermediate oxygen and cytokines.

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One of the cytokines that play a role in the pathology of SLE disease is TNF-α [5] which has been shown to correlate with SLE disease activity [6].

TNF-α is a proinflammatory cytokine involved in the development of follicles at the time of ovulation, the formation of the corpus luteum, and endometrial function. However, if TNF-α increases at the time and place (wrong tissue) it will have a bad impact on the outcome of Pregnancy. Elevated levels of TNF-α in placental tissue were also reported to have an impact on poor outcomes in Pregnancy, such as IUGR, preeclampsia and premature birth. [7] TNF-α is synthesized along the female reproductive tract, in the placenta and embryo. The expression of this gene is influenced by the development of steroid hormones and lipopolysaccharides. The differential expression of the two species of TNF receptors is regulated by the steroid hormone femininity, and this partially determines the function of TNF-α. TNF- α is a powerful, multifunctional cytokine in the process of autocrine and paracrine of the reproductive center, including gametes, follicles and luteal development, steroidogenesis, cyclicity of the uterus, differentiation of the placenta, embryonic developmentbrio [7].

Based on the theory above, researchers want to prove whether an increase in alpha TNF levels can affect blood pressure, urine albumin, and fetal body weight of Balb/c Strain Mice in Pregnant SLE Models.

2. Material and methods

It was an experimental research with the Post Test Only Control Group Design method. The research was conducted at the Pharmacology Laboratory, biomedik laboratory dan anatami-fisiologi Laboratory, Faculty of Medicine, Universitas Brawijaya, and Malang in oktober 2015 - Februari 2016.

2.1. Animal Trials

The experimental animals used were female BALB/c strain mice aged 10-12 weeks, with an average body weight of 25-40 grams, white-colored, actively moving, and moving usually. These mice are used because the mechanism of developing trophoblast cells in the placenta and mammals is inline. This study was divided into two groups, each consisting of 10 Balb/c mice). One group is a pregnant group. One group is an SLE-pregnant group. Mice are in the laboratory and placed in a clean husk-based container cage with a temperature between 25-30 ºC, with a day and night cycle of 12/12. Pellet-shaped feeding is given as much as 20 gr/day/head, which is given once a day. Drinking is placed in a special bottle with a daily requirement of 60 ml/head. The treatment of mice BALB/C has passed the Eligibility of Ethics at the University of Brawijaya Malang.

2.2. Pristane- Induced SLE

SLE Pristan induction was performed only once, i.e., as much as 0.5 ml (Santa Cruz Biotechnology, USA), and injected in BALB/c female mice intraperitoneally. after 12 weeks, the mice will show clinical confectionery of SLE such as hair loss, malar rash, decreased appetite, and an increase in ANA, after which new mice are met and conceptualized.[8,9]

2.3. Breeding

Breeding occurs when the female mice are in the estrus phase. To synchronize the estrus phase of mice, the process of tailing mice is carried out through several stages the first stage is that the female mice are in the leeboot effect, namely the anestrus condition, then the next step is to re-create the estrus phase in the female mice is carried out pheromone effect. Namely, the mice are placed into a cage that the female mice have previously occupied within 72 hours. Male mice that have been in the estrus phase simultaneously (whitten effect) are then mated by combining females and males (2:1) for one night in one cage. A vaginal Plug examination is carried out in the morning. It is obtained if the vaginal plug is positive, then the day is assumed to be the zeroth day of gestation, and the female mice are separated from the male mice.[10,11]

2.4. Blood Pressure Check

Blood pressure measurement is carried out once on the 14th day of gestation by measuring the Mean arterial blood pressure (MAP).in accordance with the procedures of the Kent Scientific CODA. Mice are inserted into tubes according to the size that has been provided. After a calm squeak, the mice are placed in a holder in a calm position, and then the top is covered with a black cloth, and a blood pressure check is carried out for 15-20 minutes. [12,13]

2.5. Urine Albumin Examination

On the 15 days, urine albumin levels were checked by placing mice in a metabolic cage to accommodate urine samples. Urine is accommodated within 12 hours. Urine albumin measurements are carried out by the ELISA procedure of the mouse albumin kit (Elabscience, USA, catalog number E-EL-M0656)

2.6. Surgery, removal of placental organs, and weighing body weight of the fetus of mice

The termination is carried out on the 18th day of gestation for the fetus to be taken and considered using Mettler AE50 analytical weighing device and taken placenta organs.

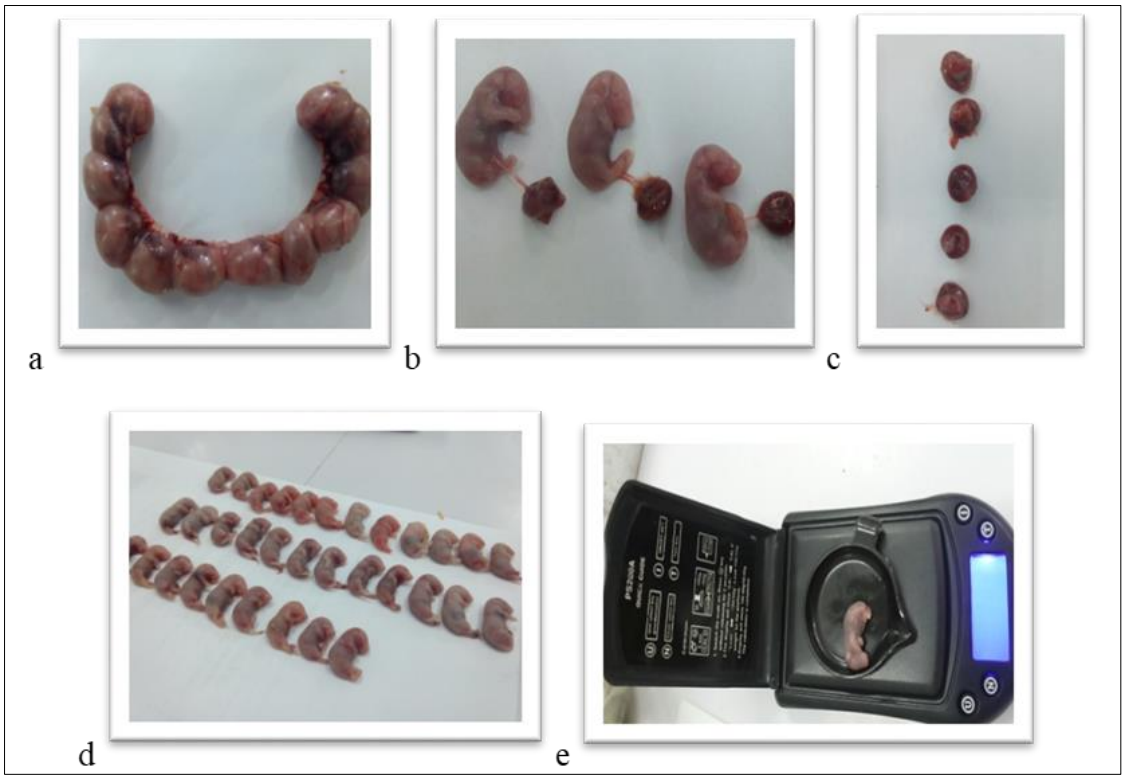

Figure 1 Measurement of length and weight of mice. a. Image of fetal mice in a pregnancy bag, b. Fetal mice and placenta, c. Placenta squeak, d. Fetal mice, and e. Weighing mice

2.7. Measurement of TNF-α Levels

2.7.1. Isolation of cytokines from placental tissue

The placenta was homogenized with twice the volume of 0.1M Tris-buffered saline (PH 7.4) hooded 0.5% Triton X-100 and one tablet of a complete mini protease cocktail inhibitor of 10 ml (Roche Diagnostics, Indianapolis, IN). Then it was centrifuged at 15,000 rpm for 30 minutes. Furthermore, the supernatant is collected and stored at a temperature of -80 C Until the Inspection is carried out. TNF-α levels are measured using the ELISA method in accordance with the guidelines listed on thekit (LEGEND MAXTM ELISA kit with pre-coated plates and Mouse TNF-α paint. No 430907).

3. Results and Discussion

After researching to determine the relationship between TNF-α levels in the lupus bunting model mice to the results of pregnancy outcomes, the following results were obtained

Figure 2 shows that the average TNF-α levels of the placenta in the treatment group (20.6 pg/mL ± 8.5 pg/mL) were higher than the average in the control group (1.16 pg/mL ± 3.6 pg/mL). After different tests were carried out using unpaired t-test analysis, the TNF alpha level of the placenta formed in the treatment group was obtained to be significantly higher than the control group with a p-value = 0.006. Because the p-value < 0.05, there is a significant difference between the treatment and control groups.

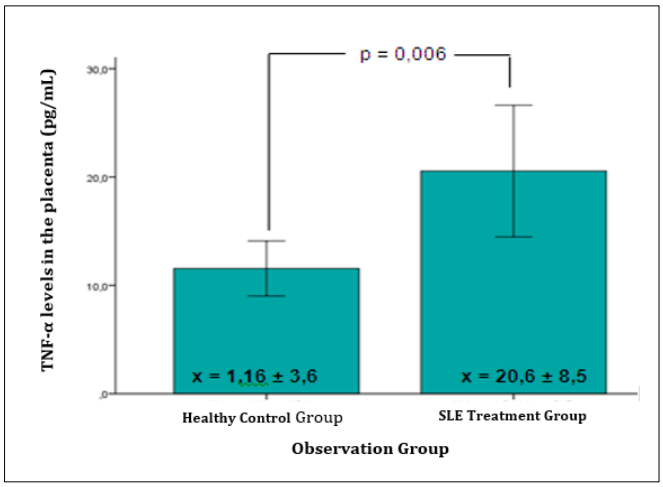

Figure 2 Graph of TNF-α Level Measurement Results

3.1. Results of Comparative Test Results of Pregnancy Outcomes in Mice Model of Lupus Bunting and Healthy Control

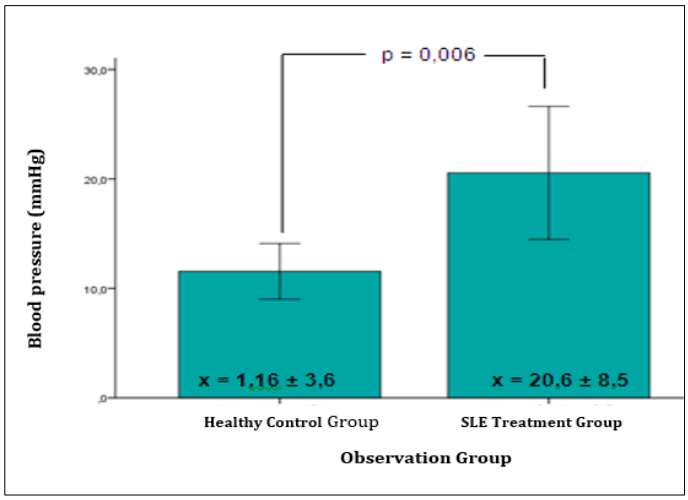

Figure 3 Average blood pressure

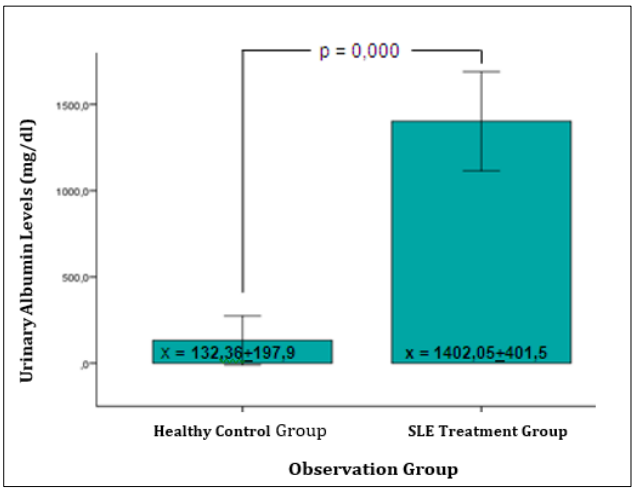

Figure 4 Average Urinary Albumin Levels

Figure 3 shows that the average blood pressure in the treatment group (91.80 mmHg ± 22.32 mmHg) was higher than the average in the control group (69.80 mmHg ± 7.62 mmHg). Furthermore, after different tests were carried out using unpaired t-test analysis, the blood pressure formed in the treatment group was obtained to be significantly higher than the control group with a p-value = 0.013. This suggests that there are significant differences between the treatment group and the healthy control group.

Figure 4 showed that the average urine albumin levels of the treatment group (132.36 mg/dl ± 197.97 mg/dl) were higher than the average in the control group (1402.05 mg/dl + 401.53 mg/dl). After different tests werecarried out using unpaired t-test analysis, the level of urine albumin formed in the treatment group was obtained to be significantly higher than the control group with a p-value = 0.000. This suggests that there were significant differences in urine albumin levels between the treatment group and the healthy control group.

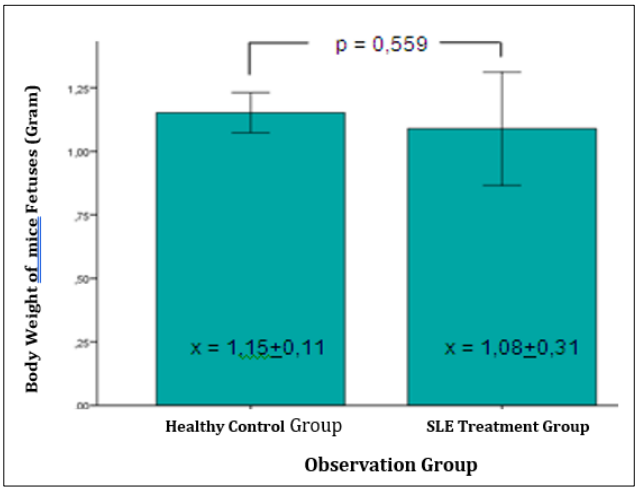

Figure 5 Average Body Weight of Mice Fetuses

Based on figure 5 above, the average body weight of the mice fetus obtained from the treatment group (1.08 Grams + 0.31 Grams) there was no significant difference from the control group (1.15 Grams + 0.11 Grams) where the p-value = 0.559. This shows that there is no meaningful difference between the treatment group and the healthy control group

3.2. Correlation of TNF-α Levels of the Placenta with Blood Pressure, Urinary Albumin, and the West of the Fetal Body

Table 1 Correlation of TNF-α with blood pressure, urinary albumin, and fetal weight

Cytokines	Output Results	Correlation coefficient	P-Value
TNF- α	Blood pressure	0,002	0,994
	Urinary albumin	0,564	0,01
	Fetal Weight	-0,176	0,458

P Value Singnifikan If <0.005

Table 1 above shows the result of the absence of a meaningful correlation between TNF-α levels of the placenta and blood pressure of BALB/C mice, which is indicated by the significance value p = 0.994 (p>0.05). This is also indicated by the degree of closeness of the relationship or the value of the correlation coefficient, which is very weak and 0.002.

It can also be seen in table 1 above after a Pearson correlation test was carried out in both groups, the value of r = 0.564 was obtained with a significance value of p = 0.01 (p<0.05). This shows that there is a meaningful correlation between TNF-α levels in the placenta and urinary albumin levels with a moderate degree of relationship tightness or correlation coefficient value of 0.564.

Then table 1 above also shows the results of the absence of a meaningful correlation between TNF-α levels of the placenta and the fetal body weight of BALB/c mice. This is indicated by the significance value p = 0.458 (p>0.05). This

is also indicated by the degree of closeness of the relationship or the value of the very weak correlation coefficient, which is -0.176.

3.3. Differences in Tumor Levels of Factor-Alpha Necrosis (TNF-α) placenta in SLE mice and Healthy Control

This study proved that there were significant differences in TNF-α levels between the control group and the treatment group. The TNF- α in the placenta in the experimental animal group of lupus bunting models, was higher than in healthy controls (p = 0.006). This is in line with other studies that state that there is an increase in TNF-α levels in the serum of patients with active SLE, in addition to TNF-α levels that have been shown to correlate with SLE disease activity. [14]

Systematic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease with an unknown aetiology and diverse clinical manifestations.[15] TNF-α is a proinflammatory cytokine that plays a lot of role in immune regulation [16]. Based on Calleja et al. in their research explained that TNF-α is synthesized along the reproductive tract of women, the placenta, and the embryo and plays a role in the development of follicles at the time of ovulation and regression of endometrial function. In addition, TNF-α has a dual role in Pregnancy; in early Pregnancy at normal levels, TNF-α is necessary for trophoblast invasion, while an increase in TNF-α by macrophages can facilitate the occurrence of childbirth in and of Pregnancy. [7,17]

Another study conducted by Pamela et al. states that the placenta is very sensitive to proinflammatory cytokines. This statement is supported by Asdown et al. (2006), which state that TNF- α levels are found to increase in the serum of the placenta mice induced innate immune as in SLE disease. [18][19]. If the cytokines TNF-α are produced excessively, it can impact tissue damage.[6]

Based on the explanation above, it can be concluded that the results obtained in this study are in line with the theory and research conducted previously.

3.4. Differences in blood pressure, urine albumin, and fetal weight in BALB/c mice in pregnant SLE models

In this study, it was found that Pregnancy with SLE can result in an increase in blood pressure and an increase in urinary albumin, which is one of the complications that can cause preeclampsia. Based on the analysis of data that has been carried out, it is proven that there is a significant difference between urine albumin levels in the mice bunting of the control group and treatment. Higher urine albumin levels were obtained in the treatment group compared to the healthy control group with a p-value = 0.000. Meanwhile, in blood pressure measurement, higher average blood pressure was obtained in the treatment group compared to the control group with a p-value = 0.013.

One of the potential factors that can result in a high prevalence of hypertension in SLE patients is due to increased levels of inflammatory cytokines such as TNF-α, IFN-γ, and IL-6 involved in pathogenesis. Other study results reported a direct link between circulating inflammatory cytokines (TNF-α and IL-6) and blood pressure in patients with hypertension [20]. The theory above follows this study, where an increase in inflammatory cytokines is found, namely TNF- α, which can be related to an increase in blood pressure of Balb / c mice in pregnant SLE models

Thirty-seven pregnant women conducted another supportive study with SLE, which found that 19.4% of preeclampsia cases assessed based on an increase in blood pressure and proteinuria after 20 weeks of gestation. In his research, it was found that kidney disorders are predictors of preeclampsia. This follows the study of Nossent et al. (1990), which reported that cases of preeclampsia in Pregnancy increased in SLE pregnant women with renal impairment.

In this study, the indicators of urine albumin in Balb/c mice modeled on lupus (pristane induction) bunting were higher when compared to healthy control mice. Following the explanation above, it is assumed that a disturbance causes the increase in urinary albumin in lupus bunting mice at the level of the renal glomerulus. Research conducted by Satoh et al. found that Balb/c mice injected with pristane intraperitoneally showed an increase in autoantibodies and the presence of significant proteinuria. [21]

Pristane induces an immune complex that mediates the occurrence of glomerulonephritis in BALB/c mice, in the presence of glomerular deposits of IgG and complements, cell proliferation, and proteinuria. Mice C57BL/6 also develop into mild nephritis (mesangial, Class II). In BALB/c mice, deposition of mesangial immune complexes is followed by subendothelial lesions consistent with diffuse proliferative (Class IV) lupus nephritis. IL-6, IFN-γ, and IL12p35 receptor deficiencies are highly resistant to kidney disease. IFNa/b receptor deficiency also decreases the incidence of proteinuria but does not alter the deposition of immune complexes in the glomerulus after being injected pristane [22].

Based on this study, it was also found that the average fetal weight of the group's mice bunting and the treatment of lupus-model bunting mice and healthy controls statistically showed no significant difference (p=0.559). Although judging from the average body weight of the fetus, there was a decrease in the treatment group compared to the healthy control group. This is inconsistent with research that has been carried out by Clowse (2006), which says that the risk of BBLR and IUGR is greater in pregnancies with SLE [23]. The ischemia/hypoxia of the pkasenta can interfere with the flow of fetal blood from the placenta so that fetal weight is lost. This is also inconsistent with a study conducted by Branch et al. (2000) which found that 23% of the 86 pregnancies with SLE experienced low birth weight. Based on this study, it can be concluded that the condition of lupus severity (flare) and stress experienced by the mother can affect the weight of the fetus due to aggression from the placenta. [24]

3.5. Relationship of Placental TNF-α levels to blood pressure, urinary albumin, and fetal weight in BALB/c mice in pregnant SLE models

Based on the results obtained in this study, it was proven that pristane injection in the BALB/c mice treatment group model of lupus bunting. May increase blood pressure compared to the control group. Based on the difference in t-tests, the results were obtained that the average blood pressure in the BALB/c mice treatment group of the pregnant SLE models was higher than that of the control group (p=0.013), as well as an increase in urinary albumin levels (p=0.000).

In this study, an increase in TNF-α levels at an inopportune time can negatively affect Pregnancy. TNF-α levels that exceed normal levels are associated with an increase in blood pressure due to endothelial dysfunction, which results in occlusion of blood vessels, reduces regional blood flow, and increases endothelium permeability. [7,25,26]. The study is in accordance with the results of this study where lupus mice (pristane induction) bunting occurs an increase in blood pressure and is accompanied by an increase in urine albumin, which is associated with a disorder in the kidneys. [1]

The exact mechanism of how inflammation causes hypertension is still unknown. Hypertension is associated with changes in the structure of arterial resistance, and it is mediated by several factors, such as angiotensin II (Damas et al. 2001). Angiotensin (Ang) II is a regulatory hormone that is formed in several tissues and has an important role in blood pressure. A decrease in glomerulus filtration rate (GFR) due to kidney disorders will be able to cause an increase in the concentration of Ang II 1000 times higher than in blood circulation, so it will increase blood pressure in the capillary glomerulus caused by the narrowing of the arterioles. Ang II will then bind to receptors throughout the body, and the effect caused to the vascular system is to increase the constriction of blood vessels and increased blood pressure.[26,27]

In this study, the decrease in GFR function obtained through the results of the examination of urinary albumin levels in SLE mice was associated with an increase in Ang II and resulted in an increase in blood pressure. Ang II will also activate NF-kB, a transcription factor that regulates gene expression for proinflammatory cytokines on vascular cells through AT1 and AT2 receptors.

Pregnancy hormones also influence the occurrence of hypertension. However, estrogen is generally considered a protector against cardiovascular disease, and its immunomodulatory properties can contribute to an increased risk of hypertension and cardiovascular disease found in young women and pregnant women with SLE.[26] In this study, results were obtained that there was a significant increase in urine albumin in the BALB/c mice model of lupus (Pristan induction) bunting compared to the healthy control bunting mice group. Such a significant increase is likely to occur an increase in inflammatory cytokines caused by the rise in TNF-α and IFNγ through pristane induction. The presence of immune complexes deposition results in the activation of complements and inflammatory damage of tissues in the kidneys. This is suspected to be the cause of high levels of urine albumin in Pregnancy with SLE.

Based on the theory and research mentioned above in accordance with the results of this study, wherein the BALB / c mice SLE pregnant models. There was an increase in blood pressure accompanied by an increase in urine albumin, which was associated with a disorder in the kidneys In addition to that in this study there was also a relationship between TNF-α levels and blood pressure with a weak level of tightness. Based on the theory described earlier, researchers assume that the theory corresponds to this theory. Increased TNF-α is an inflammatory cytokine associated with increased blood pressure and urinary albumin.

In this study, results were also found that there was no meaningful relationship between the increase in TNF-α levels and fetal body weight in the mice bunting of the healthy control and treatment groups. In addition to TNF- α, there are many factors that can affect the body weight of the fetus. The presence of disorders in the placenta can cause the occurrence of placental hypoxia, which can cause IUGR [28].

4. Conclusion

In this study, it can be concluded that there is a significant relationship between TNF- α the placenta to urinary albumin while blood pressure and fetal weight are not significantly related.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We warrant that the article is the Authors' original work and ensure no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is not under review at any other publication.

Statement of ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards at the University of Brawijaya, Malang, numbered 328/ EC/KEPK/05/2015.

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