

Intraperitoneal Injection of High Tumor Necrosis Factor (TNF- α) Serum Increase Soluble Fms-like Tyrosine Kinase 1 (sFlt-1) and Blood Pressure of Pregnant Mice

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ABSTRACT

Preeclampsia has major symptoms of hypertension and proteinuria and is a cause of significant maternal and infant mortality in the world. The slow development of preeclampsia research possibility created by the difficulty in acquiring animal preeclampsia. Many existing animal model have been developed, but most of them are expensive to do. The purpose of this study was to determine the effects of intraperitoneal injection of pregnant patients serum with high TNF- α levels toward sFlt-1 serum concentration and blood pressure of pregnant mice. Pregnant patients serum with high TNF- α levels (>20 pg/mL) was injected intraperitoneally to pregnant mice at gestational age 13 and 14 days. At 18 days of gestation, the blood pressure was measured, then the mice were dissected and the serum was taken to measure serum sFlt-1 concentration using ELISA kit (Bioassay Technology Laboratory, E0611Mo). The results showed there was a significant increase in blood pressure ($p=0.000$) and the sFlt-1 levels ($p=0.002$) of injected pregnant mice group compare to control group. These finding demonstrated that intraperitoneal injection of pregnant patients serum with high TNF- α levels to pregnant mice can increase blood pressure and sFlt-1 serum concentration of mice.

Keywords: blood pressure, preeclampsia, sFlt-1, TNF- α

INTRODUCTION

Preeclampsia is a syndrome in pregnancy that occurs in over 20 weeks of gestational age, with hypertension and proteinuria as the main symptoms [1]. Preeclampsia is a cause of maternal and infant mortality in the world and also can lead 12-25% occurrence of fetal growth restriction. The incidence of preeclampsia ranges from 3% to 10% of pregnancies worldwide, and the impact of preeclampsia is more prevalent in developing countries [2].

Preeclampsia is preceded by the disruption of the placental invasion of the endometrium [3]. These conditions will cause placental hypoxia that can lead the placental damage, followed by high inflammatory and immune responses in the placenta. Activated immune responses will result in producing the angiotensin II type 1 receptor autoantibodies (AT1-AA) which then synthesize TNF- α [4]. Tumor necrosis factor α (TNF-

α) induces the formation of high antiangiogenic factor levels in serum, one of which is soluble fms-like tyrosine kinase-1 (sFlt-1).

The sFlt-1 is a vascular endothelial growth factor receptor 1 (VEGFR-1) or Flt-1 that lack of transmembrane and a cytoplasmic domain. The sFlt-1 is not bind on the plasma membrane of the endothelial cell but soluble in the serum. This lack of transmembrane and cytoplasmic domain make the sFlt-1 can not continue the second messenger of this receptor. [5]. The increasing of sFlt-1 serum levels resulting in decreasing serum levels of it is a ligand, the vascular endothelial growth factor (VEGF), and placental growth factor (PlGF). The decreasing of VEGF and PlGF will cause clinical symptoms of preeclampsia like hypertension and proteinuria [6].

One of the preeclampsia treatments is by giving magnesium sulfate to prevent seizures. However, this treatment does not treat or decrease the progression of preeclampsia [1, 7]. The slow progress of research in finding therapies preeclampsia likely influenced by the difficulty of obtaining experimental preeclampsia animal models. A previous study revealed that injecting

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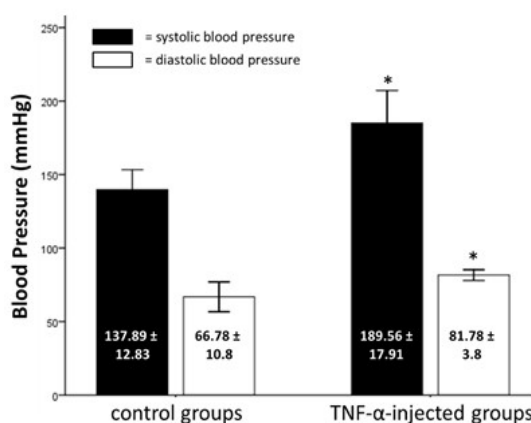


Figure 1. Pregnant Mice Blood Pressure. Serum with high TNF- α levels was injected intraperitoneally to pregnant mice at gestational age 13 and 14 days. Mice blood pressure was measured at gestational age 18 days. Injection of serum with high TNF- α levels to injected mice group resulted in increased of systolic and diastolic blood pressure compare to control group (*showed significantly different, $p < 0.05$)

TNF- α in pregnant mice will lead to hypertension and proteinuria [8].

Hence in this study we want to know the effects of intraperitoneal injection of pregnant patients serum with high TNF- α levels toward sFlt-1 serum levels and blood pressure of pregnant mice. We also want to know whether this study method possibility can be used as preeclampsia mice model.

MATERIALS AND METHODS

Experimental Design

This research is a purely experimental (true experimental) laboratory study using a posttest only controlled group design. This study was performed in vivo to determine the effects of intraperitoneal injection of pregnant patients serum with high levels of TNF- α (>20 pg/mL) toward sFlt-1 serum concentration and blood pressure in pregnant mice.

Experimental Subjects

This study used pregnant patients as serum sources and pregnant mice as an experimental subject. Pregnant patients were patients who control to Obstetrics Department of dr. Saiful Anwar Hospital at age 28-35 years and 30-40 weeks gestation with an agreement to participate in this study. Serum was collected from pregnant patients serum with TNF- α levels more than 20 pg/mL. Patients with infectious diseases and other chronic diseases are excluded from this study. Pregnant mice were used as many as 18 mice and were divided into two groups (each group consists of nine mice). One group was not injected and included as a control

group, and the other group was injected with the serum of pregnant patients with high TNF- α levels (TNF- α group). This study was approved by the Ethics Committee of the Faculty of Medicine, University of Brawijaya.

Pregnant Patients TNF- α Serum Levels Measurement

Blood was drawn from a pregnant patients vein using five cc syringe and put in plain vacutainer. Blood stored in a refrigerator at 4°C for 12 hours and then centrifuged at 6000 rpm for 10 minutes. Serum was taken and measured for the TNF- α levels using ELISA kit (BioLegend, USA, catalog number 430207). Serum with high levels of TNF- α then grouped and homogenized by the strong mixture. After measuring TNF- α levels in the serum with ELISA, we found that the mean of TNF- α serum levels from six pregnant patients were 27.59 ± 5.39 pg/mL.

Serum Intraperitoneal Injection

Pregnant patients serum with high TNF- α levels (0.1 cc) was injected to pregnant mice at the age of 13 and 14 days of pregnancy [4, 9]. The Serum was injected on intraperitoneal of the upper right abdomen with a slope of 45° and 0.5 cm deep with one cc syringe and 27G needle.

Blood Pressure Measurement

Mice blood pressure was measured at the age of 18 days of pregnancy before dissected. Mice were placed on mice stabilizer then mounted by the cuff and pulse recorded on the tail. Mice non-invasive blood pressure measuring machine (UgoBasile, Italy, product number 58500) was stand run to measure systolic and diastolic

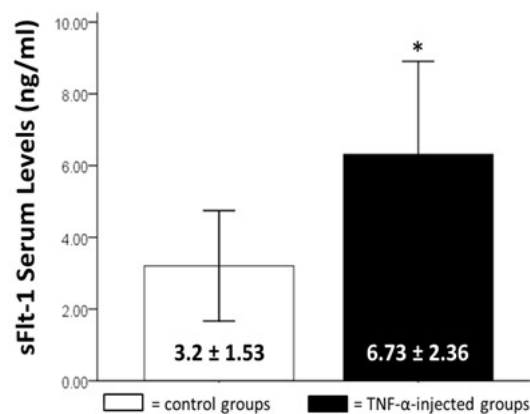


Figure 2. sFlt-1 Serum Levels of Pregnant Mice. Serum with high TNF- α levels was injected intraperitoneally to pregnant mice at gestational age 13 and 14 days. Mice was dissected at a gestational age of 18 days, and the serum was taken from the heart. Injection of serum with high TNF- α levels to injected mice group resulted in increased of sFlt-1 serum levels compare to control group. (*showed significantly different, $p < 0.05$)

blood pressure of mice. The measurement results were analyzed using independent t-test analysis.

Mice sFlt-1 Serum Levels Measurement

Mice were dissected at the age of 18 days of pregnancy using chloroform. Mice blood was taken directly from the right heart using one cc syringe and 27G needle. Blood was stored in a refrigerator at 4°C for 12 hours and then centrifuged at 6000 rpm for 10 minutes. Serum was taken and measured the sFlt-1 levels using ELISA kit (Bioassay Technology Laboratory, China, catalog number E0611Mo). The measurement results were analyzed using independent t-test analysis.

RESULTS AND DISCUSSION

Mice Blood Pressure

We injected serum with high TNF- α levels to the injected mice group as described above and measured their systolic and diastolic blood pressure. Systolic and diastolic blood pressure increase in the group of mice injected with serum with high TNF- α levels. The results of measurements of systolic and diastolic blood pressure can be seen in Figure 1.

This study used independent t-test analysis ($p < 0.05$) on systolic and diastolic blood pressure. There was a significant increase in the systolic blood pressure in mice group injected with serum with high TNF- α levels compare to control group (189.56 ± 17.91 vs. 137.89 ± 12.83 ; $p = 0.00$). The diastolic blood pressure also significantly increase, and there was a significant difference between mice group injected with serum with high TNF- α levels and control group (81.78 ± 3.8 vs. 66.78 ± 10.8 ; $p = 0.01$).

Mice sFlt-1 Serum Levels

We injected serum with high TNF- α levels to the injected mice group as described above and measured their sFlt-1 serum levels using ELISA kit. Mice sFlt-1 serum levels increase in the group of mice injected with serum with high TNF- α levels. The results of measurements of serum levels of sFlt-1 mice can be seen in Figure 2. This study used independent t-test analysis ($p < 0.05$) on sFlt-1 serum levels. There was a significant increase of sFlt-1 serum levels in mice group injected with serum with high TNF- α levels compare to control group (6.73 ± 2.36 vs. 3.2 ± 1.53 ; $p = 0.00$).

This study showed that intraperitoneal injection of serum with high TNF- α levels may increase sFlt-1 serum levels of pregnant mice. The TNF- α in the serum may bind with mice TNF- α receptor. This binding stimulate the formation of hypoxia-inducible factor 1 (HIF-1) by increase the transcription of HIF-1 subunit, the HIF-1 α [10]. The HIF-1 acts as a transcription activator protein of sFlt-1 [11]. The HIF-1 is consisted of HIF-1 α and HIF-1 β subunits. The HIF-1 β is an HIF-1 subunit expressed in constant, whereas HIF-1 α is an HIF-1 subunit that normally expressed stably in hypoxic conditions by blocking its breakdown mechanism. To perform its functions as activating transcription of a gene, HIF-1 α and HIF-1 β should form dimers into HIF-1 [12]. In a normal oxygen condition (normoxia) HIF-1 α is hydroxylated, acetylated, polyubiquitinated and braked down by the proteasome. All of that mechanism is oxygen dependent. Therefore, the amount of HIF-1 α is low and can not bound with HIF-1 β to become HIF-1 dimer [13]. In conditions of high inflammatory factors, such in high levels of TNF-

α , HIF-1 α can be synthesized with higher numbers despite normoxia. The TNF- α will activate NF κ B, a transcription factor that has a downstream signal to HIF-1 α gene transcription so that HIF-1 α will be synthesized more and expressed stably [14]. The high number of HIF-1, sFlt-1 synthesis will be increased.

Tumor necrosis factor α (TNF- α) will also increase IL-6, which is the product of TNF- α downstream signal. The previous data showed that there is higher levels of TNF- α and IL-6 in patients with preeclampsia compare to normal pregnant patients [15, 16]. The high levels of IL-6 will induce the differentiation of T cells into Th17 cells and followed by decreasing in the regulatory function of Treg cells that would reduce the ratio of Treg: Th17 [17]. This condition will activate response and differentiation of B cells into B cells CD19+ CD5+. These type of B cells are autoreactive cells that can secrete high angiotensin II type 1 receptor agonistic autoantibodies (AT1-AA) in patients with preeclampsia [18]. The AT1-AA that is formed will bind and activate angiotensin 1 receptor (AT1). The activated AT1 receptor will further increase the production of TNF- α and forming more sFlt-1 [4].

This study also showed that intraperitoneal injection of serum with high TNF- α levels will increase blood pressure pregnant mice. Increased blood pressure is caused by high levels of sFlt-1 that will decrease angiogenic factors, such as VEGF and PlGF [6]. Decreased VEGF and PlGF will reduce the bond between VEGF to VEGFR-1 and VEGFR-2, and the bond between PlGF with VEGFR-1. Reduction of VEGFR-2 activation will reduce activation of eNOS that have an effect on the reduction of NO synthesis [19]. Nitric Oxide (NO) is a natural vasodilator blood vessels and serves to maintain the permeability of blood vessels [20]. Reduced NO in blood vessels will increase endothelin 1 (ET-1) which is a blood vessels vasoconstrictor, thus causing hypertension [21, 22].

Vasoconstriction that occurs in blood vessels due to the presence of ET-1 will cause endothelial dysfunction or damage to the endothelium [22]. Endothelial dysfunction that occurs in the kidneys will cause damage to the kidney glomerulus, thus causing proteinuria [23]. Endothelial dysfunction that occurs in the placenta will cause disruption of placental vascularization [24]. Disruption of placental vascularization will aggravate the condition of preeclampsia because it will increase the damage to the placenta and increase the placenta inflammatory condition [25].

This research has shown the possibility of pregnant patients serum with high TNF- α levels injected into

pregnant mice can mimic preeclampsia condition such hypertension and increase sFlt-1 serum concentration. However, some limitations are also found in this study. In our present study, we did not evaluate urine protein levels of mice that indicate proteinuria which also the main symptom of preeclampsia. Moreover, we did not evaluate the mechanism of how pregnant patients serum with high TNF- α levels can cause hypertension and increase of sFlt-1 serum concentration. Measurement of VEGF and PlGF serum concentration may be useful to explain that mechanism. Therefore, this does not rule out the possibility of opening further research in the future to cover the limitations of this study.

CONCLUSIONS

The results of this present study indicate that pregnant patients serum with high TNF- α levels that injected into pregnant mice can increase blood pressure and sFlt-1 serum concentration of mice. Thus, our present study may be developed as an experimental mice model for preeclampsia.

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