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Bodyweight and Body Length of Foetus in Endothelial Dysfunction Model Pregnant Mus musculus as Preeclampsia Induction Which Was Given Mild Regular Exercise

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Abstract

Preeclampsia is condition with high diastol blood pressure $\geq 90$ mmHg and proteinuria in $\geq 20$ weeks of gestation. Preeclampsia was the second cause of maternal mortality in Indonesia. Preeclampsia is signed by endothelial dysfunction. Prevention of preeclampsia had not been developed yet. Exercise is activity which can induce endogen anti inflammation and antioxidiant. So, it can be used to prevent the same process in preeclampsia. The goal of this research was analyzing the difference of bodyweight and body length in endothelial dysfunction pregnant Mus musculus which was given mild regular exercise and without exercise. This research used experimental with post test only with control group design, consisted of 2 steps. Step 1 to know the dose of anti QA2, and step 2 to know the effect of mild regular exercise to bodyweight and length of foetus. Step 2 consisted of 4 groups (K1 for normal pregnant, K2 for endothelial dysfunction model pregnant, K3 for endothelial dysfunction model pregnant with mild regular exercise since early pregnant, K4 for endothelial dysfunction model pregnant with mild regular exercise since 1 week before pregnant). The number of Mus musculus were 6/group based on Federer formula. Determination of anti QA2 dose to induce endothelial dysfunction was done by using some doses and examining vasoconstriction of vessel in uterus by HE examanation. The dose that caused optimal vasoconstriction was 50 ng. The result was there was no significant differences of bodyweight and body length in endothelial dysfunction pregnant Mus musculus as preeclampsia induction which was given mild regular exercise and without exercise.

Keywords: Bodyweight, Body length, Endothelial Dysfunction, Exercise

Introduction

According to preeclampsia community guideline (PRECOG), preeclampsia is condition with diastol blood pressure $\geq 90$ mmHg and proteinuria in $\geq 20$ weeks of pregnant (Milne et. al., 2005). Mechanism of preeclampsia related to oxidative stress, inflammation, and placentation (Genest et. al., 2012). It causes endothelial dysfunction which is marked by decrease of vasodilatation, proinflammation, and prothrombine dysfunction (Rajendran et. al., 2013; Widmer dan Lerman, 2014; Sancheez-Aranguren et. al., 2014).

Preeclampsia model can be made by making endothelial dysfunction with injection of anti QA2 (anti Human QA Lymphocyte Antigen 2 Region) to block placental QA2 expression which is homolog with human leucocyte antigen-G (HLA-G) expression in human. Low HLA-G in trophoblast was the predictor od endothelial dysfunction in preeclampsia (Sulistyowati et. al., 2010). Animal model which was injected by anti QA2 had higher urine protein than normal pregnant animal (Noor et. al., 2018).

Effects of preeclampsia into foetus were low birth weight, retardation of foetus growth, and intra uterine fetal death (Matthiesen et. al., 2005). Margareth et. al. (2014) stated that baby birth weight from preeclampsia mother was lower than not preeclampsia mother. Zuhrina in Margareth et al. (2014) stated that preeclampsia had risk to make low birth weight as 34%. One of the indicators that can evaluate foetus growth were bodyweight and bodylength.

Effective management for preeclampsia should be prevention since early pregnant. Preeclampsia...
preventions that ever suggested were bedrest, regular exercise, reducing salt consumption, antioxidant, diuretic, progesterone, nitric oxide (NO), calcium and aspirine (Moura et. al., 2012). Regular exercise could increase cardiorespiration function that could be suitable for hypertension such as preeclampsia (Moura et al., 2012). Mild regular exercise was exercise with low intensity and burden. Hopps et al. (2011) stated that regular exercise or combination between regular exercise and antiinflammation could decrease proinflammation. Proinflammation could induce endothelial dysfunction.

Mild regular exercise could increase interleukin 6 (IL6) that would induce interleukin 10 (IL10) (Gleeson, 2007). It also increased endogen antioxidant (Berzosa et al., 2011). Those mechanism could be the basic mechanism of prevent endothelial dysfunction in preeclampsia. the exercise could be given since before pregnant as primary prevention, and given in the early of pregnant as secondary prevention to reduce progressivity of preeclampsia mechanism. By reducing preeclampsia mechanism, its effect to foetus could be decrease also. Therefore, this research aimed to investigate the effect of mild regular exercise to bodyweight and bodylength in pregnant Mus musculus which was injected by anti QA2 as endothelial dysfunction model to induce preeclampsia.

Literature Review

Some effects of exercise related to preeclampsia were: (Genest et al., 2012)

1. **Placental Development**
   
   Exercise for mother was good for placental and foetus growth, to launch blood circulation, and to induce mild hypoxia to make citotrophoblast proliferation. Exercise also could increase placental growth factor (PlGF) to develop placental. So, exercise balanced placental angiogenic to produce healthy foetus.

2. **Oxidative Stress**
   
   Exercise stimulated antioxidant such as Glutathione peroksidase and superoxide dismutase to reduce oxidative stress. So, that process could decrease risk of preeclampsia.

3. **Endothelial Function**
   
   Exercise induced the proliferation of endothelial cell, and nitric oxide (NO) sintase expression. NO sintase produced NO to give response to vasodilatation and endothelial function. Good endothelial function after exercise decreased blood pressure, so it could prevent preeclampsia.

4. **Immune and Inflammation Reaction**
   
   Aerob exercise could reduce inflammation dan increase anti inflammation.

Research Model

**Step 1**

The goal of first step was to confirm anti QA2 dose that could make endothelial dysfunction model in Mus musculus. Design of this research was true experimental with post test only with control group design. This research used pregnant Mus musculus, 3/group. The groups of first step research were anti QA2 dose of 10 ng, 20 ng, 30 ng, 40 ng, 50 ng, and 60 ng.

All of female Mus musculus were injected by PMSG dan HCG to syncronize oestrus cycle, anf after that the female was matted by the male 1:1. The next day, they were seperated and the female Mus musculus with vaginal plug were used for the research. Vaginal plug showed as day 0 of pregnant.

Group 1 was injected by anti QA2 of 10 ng (0,1 ml) intra peritoneal in the first day of pregnant, they were terminated in the second day of pregnant. Group 2 was injected by anti QA2 of 10 ng (0,1 ml) intraperitoneal in the first and second day og pregnant, and was terminated in the third day of pregnant. Group 3 was injected by anti QA2 of 10 ng (0,1 ml) inrapertioneal in the first and third day of pregnant and terminated in the fourth day. Group 4 was injected by anti QA2 of 10 ng (0,1 ml) intra peritoneal in the first until fourth day of pregnant and terminated in the fifth day of pregnant. Group 5 was injected by anti QA2 of 10 ng (0,1 ml) intra peritoneal in the first untill fifth day of pregnant, and terminated in the sixth day of pregnant. Group 6 was injected by anti QA2 of 10 ng (0,1 ml) intra peritoneal in the fisrt until sixth day of pregnant, and terminated in the seventh day of pregnant.
Uterine of *Mus musculus* in each group that was terminated was taken. All of the uterines were examined by histopathology examination with HE coloration to know percentage of vasoconstriction of vessels in uterine. Optimal vasoconstriction was in 50 ng dose of anti QA2, so it was used as dose for making endothelial dysfunction.

**Step 2**

The goal of step 2 research was to investigate bodyweight and body length because of mild regular exercise in endothelial dysfunction model pregnant *Mus musculus*. Design of this research was true experimental with post test only with control group design.

This research used pregnant *Mus musculus*, the same procedure with step 1. The number of *Mus musculus* were 6/group. This research had 4 groups. K1 (normal pregnant); K2 (endothelial dysfunction model by injecting anti QA2); K3 (endothelial dysfunction model and given mild regular exercise since early pregnant); K4 (endothelial dysfunction model and given mild regular exercise since 1 week before gestation).

**Protocol of Mild Regular Exercise**

- a. The speed was 14 cm/second for 15 minutes
- b. The frequency was once in two days until day 14 of pregnant
- c. The treadmill was no angle
- d. Daptation of treadmill that was used: first of all, using speed of 7 cm/second for 1 minute, 11 cm/seconds for 2 minutes, 14 cm/seconds for 15 minutes.
- e. Before exercise, *Mus musculus* must be given food and drink first

**Examination of Bodyweight And Body Length**

After termination, abdomen of *Mus musculus* was dissected to get uterine, and the foetus was taken. Each foetus was neutralized first with physiological water. Foetus was weighed by digital scale twice and was calculated the mean in gram. Foetus was placed on micrometer paper. Top of head and base of the tail was signed, and after that two of those signs was measured in cm.

**Statistic Analysis**

All of data were analyzed by normality and homogenity test. If they were in normal distribution and homogen, data were analyzed by One Way Anova with 95% of significance level.

**Data Analysis**

**Step 1**

The results of step 1 research was in Figure 1, 2, and 3.

![Figure 1: Mean of uterine vessel vasoconstriction percentage](image_url)

**Note:**

K1 (anti QA2 10 ng), K2 (anti QA2 20 ng), K3 (anti QA2 30 ng), K4 (anti QA2 40 ng), K5 (anti QA2 50 ng), dan K6 (anti QA2 60 ng)
Figure 2: Nucleus of myosit in normal arteriole wall (arrow). The shape was oval (uterine preparation; cross section; HE coloration; 1000x magnification; mikroskop Nikon H600L; camera DS Fi2 300 megapixel).

Figure 3: Nucleus of myosit in vasoconstriction arteriole wall (arrow). The shape was notched (uterine preparation; cross section; HE coloration; 1000x magnification; mikroskop Nikon H600L; camera DS Fi2 300 megapixel).

Figure 1 showed optimal vasoconstriction percentage was in 50 ng dose. So, this research used 50 ng as the dose to make endothelial dysfunction to induce preeclampsia. Anti QA2 injection was 10 ng per day intraperitoneal for 5 days. Activity of vessel constraction was signed by change of myosit nucleus structure in vessel wall. In normal condition, myosit nucleus was oval (Figure 2), but in constraction condition, myosit nucleus was notched (Figure 3).

Step 2

Step 2 research investigated bodyweight and bodylength of the groups. The results were in Table 1 and 2.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Of Foetus Bodyweight (Gram)</th>
<th>Deviation Standard</th>
<th>p Value</th>
<th>Statistic Analysis</th>
<th>Alpha Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>0.6744</td>
<td>0.3882</td>
<td>0.432</td>
<td>One way Anova</td>
<td>0.05</td>
</tr>
<tr>
<td>K2</td>
<td>0.8388</td>
<td>0.4207</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K3</td>
<td>0.5979</td>
<td>0.3301</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4</td>
<td>0.9102</td>
<td>0.2907</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Note:
K1 (normal pregnant); K2 (endothelial dysfunction model by injecting anti QA2); K3 (endothelial dysfunction model and given mild regular exercise since early pregnant); K4 (endothelial dysfunction model and given mild regular exercise since 1 week before gestation).

Table 2
Analysis of foetus bodylength in all of groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Of Foetus Bodyweight (Gram)</th>
<th>Deviation Standard</th>
<th>p Value</th>
<th>Statistic Analysis</th>
<th>Alpha Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>1.9201</td>
<td>0.4314</td>
<td>0.662</td>
<td>One way Anova</td>
<td>0.05</td>
</tr>
<tr>
<td>K2</td>
<td>2.0939</td>
<td>0.4439</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K3</td>
<td>1.9367</td>
<td>0.4972</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4</td>
<td>2.1897</td>
<td>0.3428</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All of data was in normal distribution and homogenous, so analysis used One Way Anova with 95% significance level. Table 1 showed there was no significant differences of foetus bodyweight between all of groups. Data in Table 2 also showed that there was no significant differences of foetus bodylength in all of groups.

Discussion

Step 1

Figure 1 showed percentage of vessel vasoconstriction. The optimal vasoconstriction was in K5 (dose of 50 ng). This dose could block placental QA2 expression that started endothelial dysfunction to induce preeclampsia. Every *Mus musculus* has QA2. It was a gene to protect body immunity, so natural killer cell recognized gestation as itself. If QA2 did not be expressed, natural killer cell recognized implantation as non self and induced mother's immune reaction. It recognized implantation as damage associated molecular patterns (DAMPs). This reaction continued become endothelial dysfunction to induce preeclampsia (Laresgoiti-Servitje et al., 2010).

Step 2

Table 1 and 2 showed that foetus bodyweight and bodylength had no significant differences. This fact showed that mild regular exercise had not effected to foetus growth yet. Some factors that were indicated such as type of exercise in pregnant condition. Kurniawati (2015) showed that the type of mild regular exercise that could effect to other's blood pressure in pregnant was aquarobic. It increased oxygen consumption. Its frequency was twice a week for 1 hour in the third trimester. If the blood pressure was stable, circulation to foetus was also stable and it could optimize foetus growth.

If the choice of regular exercise was still treadmill, so there must be continuing study that follow duration, angle of treadmill, and the time for starting exercise. It was similar to Tomic et al. (2013). They said that there was no significant difference in intra uterine growth restriction. In that research, the factors that were included were glucose level, frequency, duration and intensity of the exercise.

Desciptive analysis of foetus bodyweight and bodylength in Table 1 and 2 showed the different data. Mean of foetus bodyweight and bodylength in K4 (endothelial dysfunction model and given mild regular exercise since 1 week before gestation) were higher than K2 (endothelial dysfunction model by injecting anti QA2 without exercise). From those descriptive data, the exercise 1 week before pregnant could be an early initiation for balancing process by producing antiinflammation and antioxidant. If the exercise was continued untilt pregnant, it was called as chronic effect of exercise. It gave positive effect to the body. Body produced IL10 as antiinflammation and endogen antioxidant after chronic exercise. It could reduce inflammation and oxidative stress in preeclampsia. because of that process, endothelial dysfunction also reduced. So the placentation became smoother. Smooth placentation made a good circulation for the foetus and its growth became better.

Conclusion

The conclusion was there was no significant differences of bodyweight and body length in endothelial dysfunction pregnant *Mus musculus* as preeclampsia induction which was given mild regular exercise and whithout exercise.
References


