Effect of L-Arginin on Placental Hipoxia Inducible Factor-1-Alpha (HIF-1-A) Expression at Preeclampsia Mice Models

Nutria Widya Purna Anggraini1*, Sri Sulistyowati2*, Besari Adi Pramono3*, Herman Kristanto4*

1) Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/ dr. Moewardi General Hospital, Surakarta
2) Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/ Universitas Sebelas Maret Hospital, Surakarta
3) Department of Obstetrics and Gynecology, Faculty of Medicine, Diponegoro University/ Kariadi General Hospital, Semarang

ABSTRACT

**Background:** Placental hypoxia is one of the theories that cause preeclampsia. In placental hypoxia of preeclampsia, there is an increase of Hipoxia Inducible Factor 1 Alpha (HIF-1-A) expression as a response to hypoxia. L-Arginine administration through the L-Arginine-Nitric Oxide pathway is expected to improve placental hypoxia. This study aimed to examine the effect of L-Arginin on placental HIF-1-A expression at preeclampsia mice models.

**Subjects and Method:** Experimental study with parallel group post-test only design. The study was carried out at the Veterinary Medicine Faculty of Airlangga University. Thirty six preeclampsia mice models were made with intravenous injection 10ng anti-Qa-2 on day 1-4 of pregnancy. They were divided into 2 groups: (1) 18 mice without treatment (control) and (2) 18 mice were given L-Arginin 200 mg/kgBW/day on day 7-15 of pregnancy. They were terminated on day 16 then immunohistochemistry examination of HIF-1-A expression was done on the placental samples. The dependent variable was placental Hipoxia Inducible Factor-1-Alpha (HIF-1-A) expression. The independent variables were L-Arginin administration. HIF-1-A expression was measured by H-Score method. The data were analyzed by t-test.

**Results:** The mean of placental HIF-1-A expression of treatment group was lower (Mean=7.98; SD=9.35) than control group (Mean=41.95; SD=32.20), and it was statistically significant (p<0.001).

**Conclusion:** L-Arginin reduces the Placental Hypoxia Inducible Factor Alpha (HIF-1-A) expression on preeclampsia mice model.

**Keywords:** L-Arginine, HIF-1-A, Preeclampsia

**Correspondence:**
Nutria Widya Purna Anggraini. Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Sebelas Maret/ Dr. Moewardi General Hospital Surakarta. Jl Kolonel Sutarto 132, Surakarta, Central Java, Indonesia. Email: nutria_dr@staff.uns.ac.id. Mobile: 08122651819.

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BACKGROUND

Preeclampsia is one of the most common problems during pregnancy worldwide and is the leading cause of maternal and infant death (Roberts, 2013). Diagnosis of preeclampsia is established based on the presence of hypertension, proteinuria, and other organ system disorders in 20-week return (Koffreh, 2014).

In Indonesia preeclampsia remains the three highest classic causes of maternal mortality, in addition to infection and hemorrhage (Himpunan Kedokteran Fetomaternal,
In this study, the authors tried to expose the pathogenesis of preeclampsia placental hypoxia, associated with preeclampsia therapy before a manifestation or clinical symptoms. Improvement of placental hypoxia condition, with administration of vaso-dilatory nutrient supplementation, L-Arginin.

**SUBJECTS AND METHOD**

1. **Study Design**

This study is an experimental study with parallel group posttest only design. The study was carried out at The study was at Animal Experimental and Biomedical Laboratory of the Faculty of Veterinary Medicine, AirlanggaUniversity.

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2. Study Sample
Animal study using mice, with inclusion criteria are female mice (Mus musculus) Swiss strain obtained from central Veterinaria Farma Surabaya. In this study obtained female mice aged 3 months, healthy, weight 20-25 grams.

The number of samples was determined by Federer’s formula (Supranto, 2000). The number of samples was 16 (n = 16) and to avoid the decrease number of samples due to death, sickness, premature or weight loss during the study by 10%, the number of samples each group were 18, so that total sample of the study were 36 mice.

The technique or sampling method in this study were made preeclampsia mice models by giving injection of 10ng iv anti-Qa-2 (homolog to human HLA-G) on days 1-4 of pregnancy. Then divided into 2 groups, first without treatment or administration of L-Arginin (control), the second group was administered L-Arginin 200 mg/kgBW/day on days 7-15 of pregnancy. On day 16 it was terminated, and took the placental sample then examined with immunohistochemical of HIF-1-A expression. Results are assessed semi quantitatively according to the H-Score method.

3. Study Variables
The dependent variable was placental Hipoxia Inducible Factor-1-Alpha (HIF-1-A) expression. The independent variables were L-Arginin administration.

4. Operational Definition of Variables
L-Arginin was defined as essential amino acids specifically synthesized by intestinal epithelial cells with dosage 200mg/kgBW/oral/day, given at days 7th-15th of pregnancy. Placental Hipoxia Inducible Factor -1-Alpha (HIF-1-A) Expression was defined as immuno- histochemical expression hypoxisubunit of a heterodimeric transcription factor hypoxia-inducible factor 1 (HIF-1) that is encoded by the HIF-1-A gene.

5. Study Instruments
HIF-1-A expression was measured by an immunohistochemical method.

6. Data Analysis
Data were analyzed by unpaired t-test when normal data distribution, and Mann Whitney’s test when the data distribution was not normal. The statistical calculation in this study used the significance level of 0.05 (confident interval 95%).

7. Research Ethics
Ethical clearance No: 648-KE 19 October 2017.

RESULTS
A. Animal and Fetal Characteristic
Animal and Fetal Characteristic in each group. Table 1 shows the characteristic of sample. At table 2 shows mean body weight of mice after treatment L-Arginin administration, body weight of mice in case group (Mean = 29.9; SD = 2.32) was higher than control group (Mean = 27.7; SD = 3.72), ant it was statistically significant (p = 0.042).

The number of fetuses in the case group (Mean = 8.8; SD = 1.47) was higher than in the control group (Mean = 6.6; SD = 2.06), ant it was statistically significant (p = 0.002).

The mean fetal weight in the case group (Mean = 0.4; SD = 0.15) was heavier than the control group (Mean = 0.3; SD = 0.08), and it was statistically significant (p = 0.002).

The mean length of fetus in case group (Mean = 2.0; SD = 0.41) was longer than the control group (Mean = 1.6; SD = 0.41), and it was statistically significant (p = 0.005).

The mean weight of placenta in the case group (Mean = 0.2; SD = 0.05) was heavier than the control group (Mean = 0.1; SD = 0.04), and it was statistically significant (p = 0.003).
**Table 1. Sample Characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (gram)</td>
<td>36</td>
<td>22.8</td>
<td>1.72</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Number of fetus</td>
<td>36</td>
<td>6.6</td>
<td>2.06</td>
<td>4.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Weight of fetus (gram)</td>
<td>36</td>
<td>0.3</td>
<td>0.08</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Length of fetus (cm)</td>
<td>36</td>
<td>1.6</td>
<td>0.41</td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Weight of placenta (gram)</td>
<td>36</td>
<td>0.1</td>
<td>0.04</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Table 2. Mean difference after treatment L-Arginin administration between control and case groups**

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (gram)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>18</td>
<td>27.7</td>
<td>3.72</td>
<td>0.042</td>
</tr>
<tr>
<td>Case group</td>
<td>18</td>
<td>29.9</td>
<td>2.32</td>
<td></td>
</tr>
<tr>
<td><strong>Number of fetus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>18</td>
<td>6.6</td>
<td>2.06</td>
<td>0.002</td>
</tr>
<tr>
<td>Case group</td>
<td>18</td>
<td>8.8</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td><strong>Weight of fetus (gram)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>18</td>
<td>0.3</td>
<td>0.08</td>
<td>0.004</td>
</tr>
<tr>
<td>Case group</td>
<td>18</td>
<td>0.4</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td><strong>Length of fetus (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>18</td>
<td>1.6</td>
<td>0.41</td>
<td>0.005</td>
</tr>
<tr>
<td>Case group</td>
<td>18</td>
<td>2.0</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td><strong>Weight of placenta (gram)</strong></td>
<td>18</td>
<td>0.1</td>
<td>0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>Case group</td>
<td>18</td>
<td>0.2</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

**B. Placental Hipoxia Inducible Factor -1-Alpha (HIF-1-A) Expression**

**Table 3. HIF-1-A placental expression in each group**

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIF-1-A placental Expression (per 10 field of view)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>18</td>
<td>41.95</td>
<td>9.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Case group</td>
<td>18</td>
<td>7.98</td>
<td>32.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 showed the mean difference of HIF-1-A placental expression in both case and control groups. Table 3 showed that HIF-1-A placental expression in the case group (Mean= 7.98; SD= 9.35) was lower than in the control group (Mean= 41.95; SD= 32.20), and it was statistically significant (p <0.001). Comparison of HIF-1-A placental expression among the study groups was also shown in Figure 1.
**DISCUSSION**

1. **Characteristics of the sample**
   The number of fetus in the treatment group (Mean= 8.8; SD= 1.47) was higher than in control group (Mean= 6.6; SD= 2.06), with p= 0.002. This is in accordance with previous studies on pigs and sheep. A study by Gao (2011), showed that pigs receiving L-Arginine the number of live-born pigs much more than control group, this is possible because, administration of L-Arginine increases amino acids, as well as the production of estrogen and progesterone hormones that improve fetal well-being conditions as well survival of the fetus. Lassala et al. (2011), shows that administration of L-Arginine does not affect the number of sheep born (Lassala et al., 2011). However L-Arginine reduces (p <0.05) percentage of overall dead birth by 23%, and increases (p= 0.050) overall percentage of live sheep by 59%. In animal studies of mice, there were no data for differences in the number of fetuses after administration of L-Arginine.

   The weight of mice after L-Arginine administration more heavier, the probable cause was in the treatment group containing more fetuses, with fetal weight also heavier than the control group. In contrast to previous study on sheep, showed that the weight of sheep with L-Aginin treatment did not differ significantly when compared to the control group (Lassala et al., 2011).

   The mean length of fetus in treatment group (Mean= 2.0; SD= 0.41) was significantly longer than the control group that (Mean= 1.6; SD= 0.41), and it was statistically significant (p= 0.005). Mean fetus weight in treatment group (Mean= 0.4; SD= 0.15) was significantly heavier than the control group (Mean= 0.3; SD= 0.08), and it was statistically significant (p= 0.002).

   This is consistent with previous research that L-Arginine, said to increase fetal weight, and improve fetal growth restriction conditions. A study by Ohta et al. (2017) reported in animal models of preeclampsia with L-Arginine administration in the mother, will improve hypertension and prevent elevated levels of endothelin-1, mRNA and sFlt-1. It also showed reduced hypertension, increased vascular function and increased
fetal weight. The administration of L-Arginine will increase fetal weight (Satterfield et al., 2013; Elizabeth et al., 2015; Chen, 2016; Verma et al., 2018).

The mean weight of placenta in the treatment group (Mean= 0.2; SD= 0.05) was significantly heavier than the control group (Mean= 0.1; SD= 0.04), and it was statistically significant (p = 0.003). Increased placental weight may be due to improvement of placental angiogenesis because the administration of L-Arginine dramatically improves placental blood flow and blood volume and increases nutrients from maternal to placenta and then into the fetus. Further increases the weight of the placenta and fetus in Animal studies of mice conducted reported results that L-Arginine increased NO concentrations and increased protein intake, this is thought to lead to increased placental weight as well as fetal weight (Roberts and Hubel, 2009; Al-Bayati, 2014).

Furthermore, active transport of the L-Arginine-NO pathway activates the carrier protein in the placental membrane microvilli causing an increase in placental weight (Ohta et al., 2017).

2. Expression of Hypoxia Inducible Factor 1 Alpha (HIF-1-A)

In the observation from immunohistochemical result was obtained the average expression of HIF-1-A group of mice models preeclampsia with L-Arginine was lower than Mouse models of preeclampsia without administration of L-Arginin group. The statistical results showed that placental HIF-1-A expression in the treatment group was significantly lower than in the control group (p <0.001). The result of the observation is in accordance with the hypothesis.

This result in according to Korkes study, that in preeclampsia HIF-1-A value increased 2.5-fold compare with no hypertensive control (Korkes et al., 2017).

Pathophysiology of preeclampsia involves maternal, fetal and placental factors. Abnormalities in placental development early in pregnancy, starting from inadequate trophoblast invasion to spiral artery, leading to decreased uteroplacental perfusion, causing placental hypoxia (Ibrahim and Khaled, 2014; Sánchez-Aranguren et al., 2014). Placental hypoxia will increase oxidative stress and activation of inducible-1α indifference pathway (HIF-1-A).

Animal studies by Ohta et al. (2017), using animals model, and to confirm placental hypoxia conditions in preeclampsia mice model, examined HIF-1-A expression in the placenta. The expression of HIF-1-A increased significantly in trophoblast, labyrinth, and spongiotrophoblast regions.

Another study Verma et al. (2018), examining the expression of HIF-1-A, was determined as a percentage with immune staining (color: golden brown chromogen) within trophoblast villi. Increased HIF-1-A expression was associated with important gene dysregulation until dysregulated function normal placenta, so increased HIF-1-A expression is an important parameter for developing a new therapeutic strategy for preeclampsia (Robb et al, 2017).

The above studies are consistent with the results of this study, in which HIF-1-A expression of placenta in the treatment group with L-Arginine administration was lower than in control group. Administration of oral L-Arginine 200mg/ kgBW/ oral day 7–15 pregnancy, because of on 7th day of pregnancy, the implantation process has been completed and the trophoblast placentation stage begins to the 15th day of the placentation process is complete (Cheryl, 2015).

In preeclampsia a prolonged hypoxia due to spiral arteria remodeling failure, HIF-1-A expression will increase and stimulate angiogenesis changes, imbalance of proangiogenic factor (VEGF and PlGF) and anti ang-
iogenic (sENG, sFlt-1). Followed by oxidative stress processes that cause endothelial dysfunction in blood vessels and other symptoms of disorders, including hypertension (Asif and Wenda, 2015). In this placental hypoxia condition, a strong vasodilator is needed to prevent prolonged hypoxia, thus preventing worsening of symptoms from preeclampsia due to oxidative stress (Robb et al., 2017).

L-Arginine, as NO precursor, is a powerful vasodilator for improving placental hypoxia in preeclampsia, especially during placentation and angiogenesis.

NO is the main vasodilator that controls blood flow to the placenta and also the fetal-placental vessels. Much evidence suggests that NO is important for maintaining a low resistance / high flow uteroplacental system that supports nutrients, fetal oxygenation (Asif and Wenda, 2015). In preeclampsia there is a decrease in activity of Nitric Oxide (NO), endothelial nitric oxide (eNOS), and a decrease in the concentration of cationic amino acid L-Arginine, which is the substrate of eNOS (Grafka et al., 2016). In Lisette’s study, Salsoso et al said NO levels decreased and ADMA increased in pregnancy with preeclampsia (Lisette, 2014).

A preclinical study conducted on mice showed that L-Arginine reduced the incidence of hypertension, in response to reduced uterine perfusion pressure in pregnant mice, suggesting that L-arginine supplementation was useful in management in the case of preeclampsia (Al-Bayati, 2014).

Other studies in rats and mice induced by preeclampsia, characterized by hypertension, proteinuria and fetal growth inhibition after administration, NG-nitro-L-Arginine Methyl Ester (L-NAME), an important inhibitor of NO synthase, improved after treatment with L-Arginine with reduced urinary protein excretion, a significant drop in blood pressure and restoration of glomeruli lesions. L-Arginine plays a role through NO synthase pathway by inhibiting nitric oxide synthase inhibitor so that NO production will increase and can give effect of vascular vasodilation and improve hypoxia (Lisette, 2014). NO from the maternal circulation will cross into the placenta and dilate the vascular placental villi by increasing the vascularity of the villi, the lumen dilatation and the muscle walls of the blood vessels so as not to have atherosis or thickening. Good blood flow perfusion will prevent the ongoing hypoxia, thus improving the condition of placental hypoxia and improvement of symptoms of preeclampsia which in this study is characterized by low expression of HIF-1-A (Wall et al., 2014).

In addition, L-Arginine modulates cellular response to hypoxia by increasing the activity of Prolyl Hydroxylase (PHD) which will degrade HIF-1-A protein so that the amount decreases (Semenza, 2011).

In this study, researchers found that the use of L-Arginine dose of 200mg /kgBW/day/orally was effective in improving placental hypoxia condition of preeclampsia mice models characterized by lower expression of placental HIF-1-A as marker of placental hypoxia in administration L-Arginine (case) group. It is also expected by L-Arginin administration, analogically will improve the symptoms of preeclampsia, and it can be analyzed that L-Arginin is a new therapy option to improve the condition of preeclampsia in human to prevent further complications.

**AUTHOR CONTRIBUTION**

Nutria Widya Purna Anggraini is the main researcher who formulated the conceptual framework, plays role in processing and collecting data of the study. Sri Sulistiyowati examined conceptual framework and methodology of the study. Besari Adi Pramono and Herman Kristanto gave the suggestion related to the discussion.
CONFLICT OF INTEREST
The authors state that there is no conflict of interest related to this study, the author, and the publication of this article.

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REFERENCE

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