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Increasing of LDH Specific Activity and PEPCK Level Play a Role on Activation of Gluconeogenesis Pathway in Early Onset Pre-Eeclampsia Placenta

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Abstract

Background: Recent advancement on experiment concluded that etiology of pre-eclampsia (PE) could be explained by the "two-stage" theory. The theory of which explained that pre-eclampsia occurs due to abnormalities in spiral arteries development and release of inflammatory response. Failure of spiral arteries development, the lesion of damage could be due to ischemia-reperfusion or hypoxia-reoxygenation. Hypoxia in pre-eclamptic placenta leads to metabolic change to anaerobic in glycolysis. Lactate dehydrogenase (LDH) has important role in anaerobic glycolysis that catalyzes the conversion of lactate to pyruvate during hypoxia. On the other hand, phosphoenolpyruvate carboxy kinase (PEPCK) is merely an enzyme of gluconeogenesis. This research conduct to reveal that in early onset pre-eclampsia the placenta still hypoxic and undergoes gluconeogenesis even after delivery, through metabolic enzyme of LDH and PEPCK level.

Methods: This cross-sectional study compared early onset PE (< 34 weeks) with normal term placenta. We measured LDH enzyme activity using colorimetric assay and PEPCK protein using ELISA method.

Results: Result show that placental LDH specific activity was increased significantly in PE with median 2.750 (0.030 - 5.680) U/mg compared to normal term placenta 0.255 (0.032 – 1.194) U/mg (Mann-Whitney, p< 0.001). PEPCK level was significantly increased in PE 8.998 (1.737-44.914) ng/mg compared to normal term placenta 1.552 (0.741-8.832) ng/mg (Mann-Whitney, p< 0.001).

Conclusions: We conclude that anaerobic glycolysis and gluconeogenesis pathway are increased in early onset PE placenta as adaptation mechanism to hypoxic condition.

Keywords: Early onset preeclampsia, LDH, PEPCK, Placenta.

Introduction

Pre-eclampsia is a disorder characterized by new onset hypertension that occurs during pregnancy. In worldwide, preeclampsia is considered as one of the major sources of mortality and morbidity. Approximately 10 – 15% of maternal deaths are related to pre-eclampsia and eclampsia (1). Almost 10% of all maternal deaths in Asia and Africa are correlated with hypertensive disorders of pregnancy (2). About 5 – 10% of all pregnancies have pre-eclampsia as a complication while 20% of who are primipara complicates to pre-eclampsia (3).

Pre-eclampsia is characterized and defined

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as a multisystem disorder with a new onset of hypertension with systolic blood pressure of more than 140 mmHg or diastolic blood pressure of more than or equal to 90 mmHg and proteinuria≥ 0.3 g for 24 hours (1). The hypertension is advised to be measured twice with at least 4 hours in between the two measurements unless it is urgent. Generally, it occurs after 20 weeks of gestation, with most cases usually shows that it occurs near term (1). In addition, maternal organ dysfunction comprises of renal insufficiency or pain in the right hypochondrium or impairment in liver function or neurological complication, all of which has their own characteristic. To be noted, that proteinuria is not a mandatory abnormality that present in pre – eclampsia (4,5).

Recent advancement on experiment concluded that etiology of pre – eclampsia could be explained by the "two – stage" theory. The theory of which explained that pre – eclampsia (PE) occurs due to abnormalities in spiral arteries development and release of inflammatory response. Due to failure of spiral arteries development, lesion of the damage could be due to ischemia – reperfusion or hypoxia – reoxygenation. The latter is caused by released of free radicals, for instance the Reactive Oxygen Species (ROS). The effect of the afore mentioned causes, lead to release of particles into the serum, trigger inflammatory responses, and imbalance between anti – angiogenic and angiogenic factors. The end result would be the malfunction of the endothelial cells, which trigger the onset of pre – eclampsia (4–6).

Due to hypoxia in preeclamptic placenta, metabolic pathway that will increase is anaerobic glycolysis. Lactate dehydrogenase has important role in anaerobic glycolysis that will catalyze the conversion of pyruvate to lactate. A study on 200 pregnant women with preeclampsia, where 121 has mild preeclampsia and 79 experiences severe preeclampsia, shows that those with severe preeclampsia manifest an increasing number of serum LDH compared to the ones with mild preeclampsia. The same study also highlights that those with serum LDH> 800 IU/L shows a significant increase in symptoms as well as complications of pre-eclampsia together with perinatal mortality (7).

LDH is a multifaceted intracellular cytoplasmic enzyme (7,8). It has 5 isoenzymes, where each has a slightly distinct structure compared to the others and the concentration of it differs in different tissues. All of its isoenzymes can be found in the placenta (8). It is increased in conditions with a higher pH and when hypoxia is present, which is the case in patients with pre-eclampsia. It increases the vascular endothelial growth factor (VEGF-A) as well as concomitantly increasing the basic fibroblast growth factor (bFGF), which is an important component of embryonic stem cell. When hypoxia occurs, the glycolytic rate also increases hence increasing the activity of LDH which leads to the catalysis the reaction of pyruvate to lactate. However, it can also induce cell death through its leakage outside of the cell (8). Otherwise PEPCK is an enzyme that increases in its activity or concentration means increased gluconeogenesis. Therefore, it is important to explore more on the effect of LDH and PEPCK in patients with pre-eclampsia. This research was conducted to assess the activity of LDH and PEPCK level in placental tissues of early onset preeclampsia compared to normal term placenta to ensure that gluconeogenesis was increased in early onset pre-eclampsia.

Materials and Methods

This is a cross-sectional study, used placental tissues with informed consent and ethical approval from The Ethics Committee of the Faculty of Medicine, University of Indonesia, no.: 1325/UN2.F1/ETIK/PPM.00.02/2019. Permission for sampling is provided by the Directorate General of Health Services RSUP National Dr. Cipto Mangunkusumo with the number LB.02 / 2.2. / 10902/2018 on September 20, 2018.

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Tissue Preparation

About 100 mg of placenta tissues were placed into new clean microtubes and added with 100 mL of Phosphate Buffer Saline (PBS) 0,01 M, and in pH 7,4. Tissues were blended by homogenizer until smooth. The homogenate was centrifuged 3000 rpm for 5 minutes and the liquid supernatant was taken and stored in freezer -20 ^{o}C for the next assay.

Measurement of Total Protein Concentration

We used the Bovine Serum Albumin (BSA) as a standard protein with several concentration as follows: 800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 12,5 µg/mL. The samples were diluted with aquadest by 50 times dilution. The absorbance of standard and sample will be read at 280 nm using spectrophotometer. Standard curve was created using the absorbance data of the BSA. We calculate protein concentration of the sample using formula generated in standard curve.

Measurement of Lactate Dehydrogenase Specific Activity

Lactate dehydrogenase (LDH) catalyze the reaction of pyruvic acid into lactate reversibly. Within this research, pyruvic acid reacts with 2,4 dinitrophenylhydrazine and produce brownish red alkaline solution, which is pyruvate dinitrophenylhydrazone. The detection of pyruvate dinitrophenylhydrazone is through measurement of its Optical Density (OD) value at 440 nm. We measured LDH activity using lactate dehydrogenase (LDH) assay kit (Elabscience®). We prepared the sample, blank (distilled water) and standard (pyruvic acid 2 mmol/L) in microtube as much 50 µL respectively. After that 0.25 mL substrate buffer was added and 0.05 mL Coenzyme I solution was added only into samples tube. We mixed it and incubated the solution for 15 minutes at 37 ^{o}C. We added 0.25 mL 2,4-dinitrophenylhydrazine into the mixture and mixed it and incubate again for 15 minutes at 37 ^{o}C. We added 2.5 mL NaOH 0.4 M into the mixture to stop the reaction. After that, the absorbance was read at 440 nm using spectrophotometer. One unit of LDH is defined as 1 gram of tissue that reacts with substrate at 37 ^{o}C for 15 minutes, which produce 1 µmol of pyruvic acid.

The calculation of LDH activity in tissue (U/g prot)

$$\frac{\{(\text{OTMD sample} - \text{OD control}) \times \text{Concentration of standard mmol/L})}{(\text{OD standard} - \text{OD blank})}$$

Protein concentration of sample (mg prot/mL)

Measurement of PEPCK Level

We measured the PEPCK level using sandwich ELISA method (Human phosphoenolpyruvate carboxy kinase / PCK1 ELISA kit, Elabscience®). This assay is carried out according to the manufacturer's instructions. This method uses 2 antibodies which functioned to detect the antigen which is the human PCK1. The ELISA plate that was used in this experiment had already been pre-coated with antibodies specific to human PCK1 enzyme. Then, the sample which contained the antigen was inserted into the wells. After that, biotinylated detection antibody specific for human PCK1 was added. It was followed by the insertion of the secondary antibody Avidin-Horseradish Peroxidase (HRP), which was specific to detect the biotinylated detection antibody. Then, chromogenic substrate was added with the HRP, these enzyme-substrate conjugate would react and produced blue color. Lastly, added stop solution to stop the enzyme-substrate reaction and the solution would turn yellow and it will be read at 450 nm by ELISA reader.

Results

This research used 53 placentas from normal term pregnancy and PE pregnancies, consist of 27 normal and 26 early onset PE. Subjects were obtained from Cipto Mangunkusumo Central Hospital and Budi Kemuliaan Hospital Jakarta, Indonesia.

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Characteristic of Subjects

We demonstrated in onset PE group has found maternal ages were 29.65±5.96 years old, gestational ages were 29.56±2.5 weeks, systole pressure range 165.27±21.32 mmHg, diastole pressure 105.03±15.97 mmHg, and proteinuria 2.35±0.63. Besides, in normal term group has found maternal ages 28.58±4.57 years old, gestational ages 39.39±1.14 weeks, with normal systole and diastole pressures and no proteinuria.

Specific activity of LDH in normal and pre-eclamptic placenta

Specific activity of LDH in normal term placenta was 0.255 (0.032 – 1.194) U/mg and in early onset PE placenta was 2.750 (0.030 – 5.680) U/mg. Data distribution of specific activity of LDH doesn't have normal distribution, therefore Mann Whitney was used for statistical analysis. We found there was a significant different of LDH specific activity between normal term and early onset PE, (Mann-Whitney, p< 0.0001), (Fig.1).

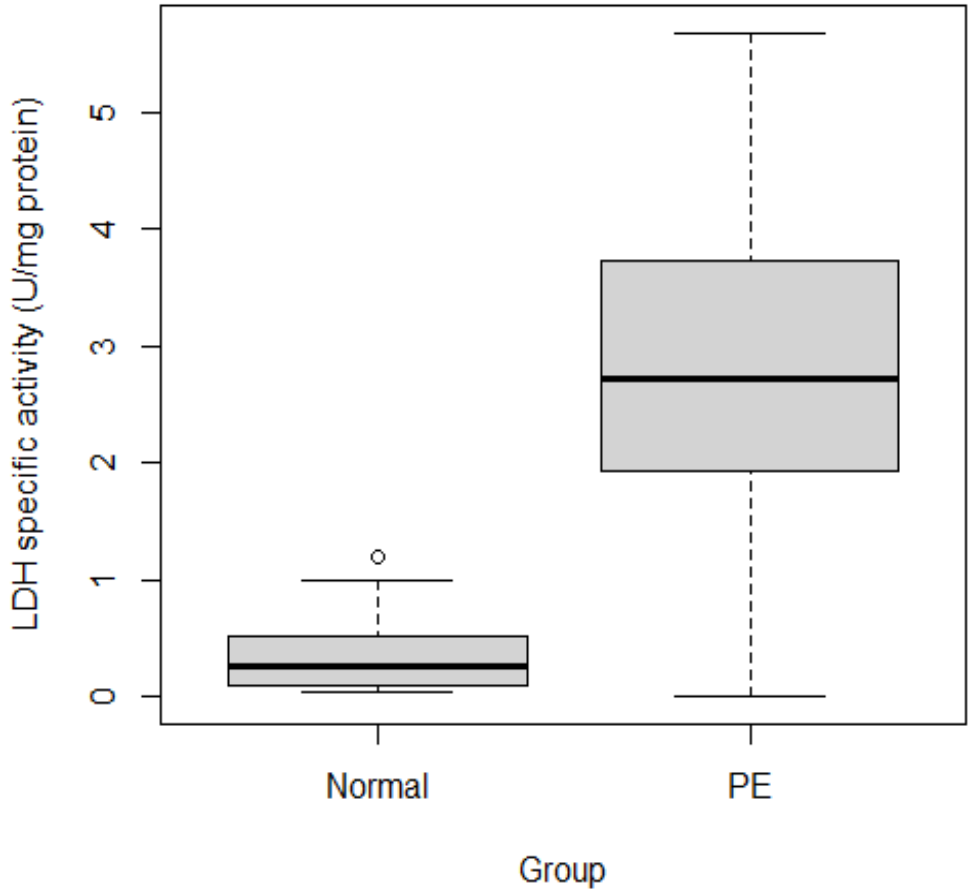

Fig. 1. The specific activity of LDH (U/mg) was increased significantly in early onset preeclamptic placenta compared to normal term placenta (Mann-Whitney, p< 0.0001).

The PEPCK level in normal and pre-eclamptic placenta

We demonstrated PEPCK concentration in normal term placenta was 1.552 (0.741 – 8.832) ng/mg and in early onset PE placenta was 8.998 (1.737 – 44.914) ng/mg. Data of PEPCK level also doesn't have normally distributed, so we analyzed their significant difference using Mann-Whitney test. We found there was a significant different between PEPCK level in normal term and early onset PE placenta (Mann-Whitney, p< 0.0001), (Fig. 2).

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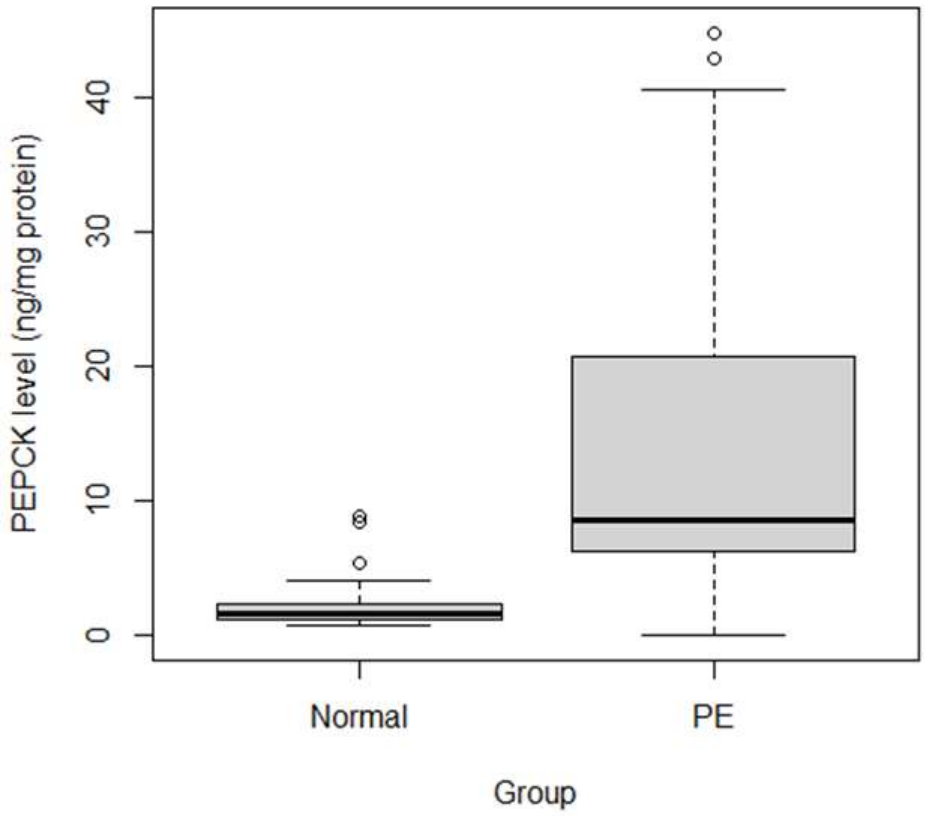

Fig. 2. Phosphoenolpyruvate carboxy kinase (PEPCK) level (ng/mg) was increased significantly in early onset preeclamptic placenta compared to normal term placenta (Mann-Whitney, p< 0.0001).

Correlation between LDH and PEPCK in normal term and early onset PE placenta

To reveal that there was an increase of gluconeogenesis in early onset PE compared to normal term, correlation between LDH and PEPCK was measured in each group using Spearman test. There was no correlation between LDH specific activity and PEPCK concentration in normal term placenta, R= 0.259, p= 0.192. On the other hand, correlation between LDH specific activity and PEPCK concentration in early onset PE placenta was nearly significant R= 0.378, p= 0.057.

Discussion

This study showed that in early onset PE placenta, the specific activity of LDH and PEPCK level were increase significantly compared to normal term placenta. Lactate dehydrogenase is the enzyme of glycolysis and also of gluconeogenesis pathway, while PEPCK catalyzes formation of phosphoenolpyruvate from oxaloacetate that is occur only in gluconeogenesis pathway (9). Lactate dehydrogenase catalyzes in two direction of lactate synthesis or pyruvate synthesis (9). Condition that determined the direction of LDH reaction are normal oxygen pressure, hypoxia or relative hypoxia condition. When tissue was under hypoxic circumstance or relative hypoxia, glucose metabolism changes to adapt against hypoxia, through becoming anaerobic glycolysis. This change of metabolism lead to increased lactate production. It also changes that part of glucose content provided only 2 ATP each, therefore in hypoxia tissues need more glucose, that supported by gluconeogenesis pathway. To proof that gluconeogenesis was increased, we measured LDH specific activity and PEPCK protein concentration. If only LDH specific activity was measured, it cannot be stated that the direction of LDH activity was to produce pyruvate, because in lactate synthesis LDH

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specific activity also increased. Therefore, to ensure the reaction direction it need measurement the level of PEPCK. In this study we found that concentration of PEPCK was increased, so it supports the reaction direction of LDH was to synthesis pyruvate to supply glucose demand in placenta.

Previous study on metabolism in pregnancies show that stress in pregnancy was associated with metabolic dysfunction. It described that saline injection-induce stress cause change of fetal metabolism in gluconeogenesis through liver PEPCK activity in fetus. PEPCK activity were increased significantly in saline injected rat, p= 0.001 in pair housed rats and p= 0.002 in single housed rats (10).

They use PEPCK and G6Pase (glucose-6 phosphatase) to measure gluconeogenesis of fetal and maternal rat liver, that stress induced with subcutaneous saline injection (0.9% w/v NaCl, 200 μL/100 g body weight). The result demonstrated that saline injection affected glucogenic capacity in maternal liver, it proved by reduce glycogen content and raise G6Pase activity. PEPCK was increased significantly in injected rats in pair housed, p< 0.001, but not in single housed. it considered that stress leads to increase gluconeogenesis. The different stress cause in Franko's study was saline injection, beside in this study we considered that stress in placenta was the failure of pseudo-vasculogenesis of spiralis artery that caused damage of syncytiotrophoblast (STB) that followed by inflammation (10,11).

Compared to this research, the stress that can triggered metabolism alteration in pre-eclampsia placenta, presumably was the hypoxia that lead to oxidative stress through measurement of glutathione, malondialdehyde, nitric oxide in PE placentas (12). In addition, systemic chronic hypoxia also lead to oxidative stress and increasing of carbonic anhydrase specific activity in rat liver (13). Stress through systemic chronic along 14 days of hypoxia also caused increased of gluconeogenesis start after 5 day long of hypoxia (14). As we know that in pre-eclampsia placenta has chronic hypoxia caused by failure of pseudo-vasculogenesis of spiral arteries and failure synthesis of VEGF and prorenin receptor (15).

We conclude that there is increasing of anaerobic glycolysis and gluconeogenesis in early onset pre-eclampsia placenta compared to normal placenta as an adaptation mechanism to hypoxic condition.

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References

1. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi J-M. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011;7:467–474.
2. Sammour MB, el-Kabarity H, Fawzy MM, Schindler AE. Prevention and treatment of pregnancy-induced hypertension (preeclampsia) with progestogens. J Steroid Biochem Mol Biol. 2005;97(5):439-40.
3. Kahnamoueiaghdam F, Amani F, Hamidimoghaddam S. Prevalence of pre-eclampsia and eclampsia risk factors among pregnant women, 2011-2013. International Journal of Advances in Medicine. 2015;2(2):128-132.
4. Gathiram P, Moodley J. Pre-eclampsia: its pathogenesis and pathophysiolgy. Cardiovascular Journal of Africa. 2016;27(2):71–8.
5. Mayrink J, Costa ML, Cecatti JG. Preeclampsia in 2018: Revisiting Concepts, Physiopathology, and Prediction. Scientific WorldJournal. 2018;2018:6268276.
6. Zhao L, Ma R, Zhang L, Yuan X, Wu J, He L, et al. Inhibition of HIF-1a-mediated TLR4 activation decreases apoptosis and promotes

[DOI: 10.61186/rbmb.11.2.320]

angiogenesis of placental microvascular endothelial cells during severe pre-eclampsia pathogenesis. Placenta. 2019;83:8–16.
7. Mary VP, Chellatamizh M, Padmanaban S. Role of serum LDH in preeclampsia as a prognostic factor – a cross sectional case control study in tertiary care hospital. Int J Reprod Contraception, Obstet Gynecol. 2017;6(2):595.
8. Ababio G, Adu-Bonsaffoh K, Narh G, Morvey D, Botchway F, Abindau E, et al. Effects of Lactate Dehydrogenase (LDH) in Preeclampsia. Clinical & Medical Biochemistry. 2017;03(2):1–6.
9. Bender DA, Mayes PA. Metabolism of Carbohydrates. Harper's Illustrated Biochemistry. 2015.
10. Franko KL, Forhead AJ, Fowden AL. Effects of stress during pregnancy on hepatic glucogenic capacity in rat dams and their fetuses. Physiol Rep. 2017;5(11):e13293.
11. Labarrere CA, DiCarlo HL, Bammerlin E, Hardin JW, Kim YM, Chaemsaithong P, et al. Failure of physiologic transformation of spiral arteries, endothelial and trophoblast cell activation, and acute atherosis in the basal plate of the placenta. Am J Obstet Gynecol. 2017;216(3):287.e1-287.e16.
12. Prijanti AR, Marissa N, Paramita R, Humaira S, Nabila EN, Wijaya AE, et al. Analysis of oxidative stress markers malondialdehyde, glutathione, nitric oxide, and prorenin level in preeclampsia placental tissues. Asian J Pharm Clin Res. 2018;11(1):158–61.
13. Ridwan R, Iswanti FC, Sadikin M. The increased of Carbonic anhydrase in liver tissue of rat induced by chronic systemic hypoxia. Acta Biochimica Indonesiana. 2018;1(1).
14. Prijanti AR, Indarwati T, Mudjihartini N, Yulhasri, Sadikin M. Performance of Gluconeogenesis in Rat Kidneys Due to Systemic Hypoxia. Adv Sci Lett. 2018;24(9):6781–6784.
15. Marissa N, Jusman SWA, Purwosunu Y, Prijanti AR. Correlation of (PRO)renin receptor and vascular endothelial growth factor expression level in third trimester of preeclampsia placentas. Asian J Pharm Clin Res. 2018;11(12):1–5.

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