High Expression F2-Isoprostan (F2-IsoP), High Sterol Regulatory Element Binding Protein-2 (SREBP-2) and Low 2-Methoxyestradiol (2-ME) On Placenta Tissue as a Risk Factor of Pre-Eclampsia

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Abstract

To date, pre-eclampsia (PE) still a problem of Maternal Fetal Medicine related to high incidence, maternal and neonatal morbidity and mortality. Pre-eclampsia is caused by pregnancy; however, the mechanism has not been established so it is still a disease of theories. This relates to differences in treatment, resulting in different ways of prevention and output of PE itself. Recently, the role of F2-IsoP, SREBP-2 and 2-ME was suspected to be very important in the mechanism of the PE. Meanwhile, the placenta acts as a source regulatory protein production, so that the material of this study was taken from placental tissue. The study objective was to prove the high F2-IsoP, high SREBP-2 and low 2-ME expression in the placenta as risk factors for PE Case control study has been conducted in the department of obstetrics and gynecology Sanglah Hospital with 62 samples in 2015. The case group consisted of 31 mothers with PE and control groups consisted of 31 non-PE mother. The study material is a placental tissue. F2-IsoP and SREBP-2 expression study was performed using immunohistochemistry and 2-ME with ELISA techniques in Pathobiology Laboratory Faculty of Veterinary Medicine Udayana University. Data were analyzed with chi square test and discriminant using SPSS. The statistical test results are presented in tabular form and narrative. In this study, it was found that high expression of F2-IsoP increased the risk of PE 4 times higher (OR = 4.44; 95% CI = 1.53 to 12.94; p = 0.005) ; high expression of SREBP-2 increased the risk of PE 8 times higher (OR = 8.19, CI95% = 2.311 to 29.073; p = 0.001) and low expression of 2-ME increased the risk of PE 5 times higher (OR = 5.23; CI95% = 1.75 to 15.55; p = 0.002). On the discriminant test, we obtained contributing risk factor for the occurrence of PE were SREBP-2, F2Isop and 2-ME (p = 0.002) respectively. Conclusion, high F2-IsoP expression, high SREBP-2 expression and low 2-ME expression in placenta were risk factors for PE. The most dominant risk factor of PE mechanism was SREBP-2.

Keywords: Pre-eclampsia, F2-Isoprostane, Sterol Regulatory Element Binding Protein-2, 2-Methoxyestradiol.

Introduction

Pre-eclampsia (PE) is a pathology of pregnancy with multisystem clinical manifestations characterized by hypertension and proteinuria at 20 weeks of gestation. This pathology of pregnancy is an issue of reproductive health related to incidence, maternal mortality rate (MMR) and perinatal mortality rate (PMR) is still high. PE is a direct cause of maternal deaths worldwide associated with severe complications such as intracerebral hemorrhage, pulmonary edema, and renal failure.

Worldwide incidence of pre-eclampsia (PE) ranges from 3-5%, tends to fluctuate by 5-10%. There are reported to be approximately 500,000 maternal deaths and 900,000 perinatal deaths per year and mostly in developing countries [1-2-3]. While in Indonesia the incidence of PE is higher, between 5-10% and increase from year to
year. At RSUP Sanglah Denpasar, the incidence of PE in 2005 was 5.83% [4] in 2006 was 6.06% [4] and in 2013 was 9.23% [5].

Until now there is no evidence about the exact cause of pre-eclampsia, so the effective therapy for pre-eclampsia is still symptomatic. The administration of drugs may reduce the occurrence of complications, but there is no firm evidence of positive benefits for maternal and child safety. With unknown of exact cause of pre-eclampsia, prevention strategies and pre-eclampsia treatment have not been effective.

Although the exact cause of pre-eclampsia is unknown but experts agree that pre-eclampsia originates from placenta with hypoxia resulting from inadequate of cytotrophoblast invasion into the spiral artery or failure of spiral arterial remodeling, leading to oxidative stress and overall endothelial dysfunction. This is related to the disappearance of clinical manifestations when the placenta is born and does not depend on whether or not the fetus is present. Conventionally the placenta in pregnancy with pre-eclampsia is suspected to have oxidative stress and produce free radicals, suggest the expression of proteins that play a role in the emergence of pre-eclampsia clinical syndrome [6].

As is well known, oxidative stress is a condition in which an imbalance between free radical production and antioxidant defense systems results in increased peroxidation lipid production and antioxidant defense systems results in increased peroxidation lipid production. Lipid peroxidation is thought to play an important role in causing endothelial function disorders and the incidence of clinical symptoms of pre-eclampsia [7-8-9]. Such increased lipid peroxidation can be measured by measurement of lipid peroxidation products in the blood, F2-Isoprostan (F2-IsoP) [10].

F2-IsoP is a good, very stable, and significantly more accurate marker of oxidative stress or lipid peroxidation in vivo, than other markers [10]. F2-IsoP is found almost in all biological fluids, however blood (plasma or serum) and urine are the most commonly used study samples because they are the easiest to get, and at least invasive. Meanwhile, studies using F2-IsoP markers as a marker of lipid peroxidation in the placenta are currently lacking and more often in blood or plasma samples [11-1].

On the other hand, the mechanism of pre-eclampsia pathogenesis is thought to be associated with hormonal factors and dyslipidemia. In conjunction with dyslipidemia, hormone estrogen in the early pregnancy causes the activation of hepatic lipase enzyme that causes changes in plasma lipid levels towards dyslipidemic conditions. Women with pre-eclampsia are said to have differences in lipid parameters and increased susceptibility to lipoprotein oxidation and cardiovascular disease [12-10-13-8]. However, the opposite is stated by Bar et al [14] that the increase in lipoprotein was not proven to be used as a predictor of pre-eclampsia [14].

On the other hand experts say there is an analogy between atherosclerotic vascular in patients with dyslipidemia with atherosclerosis on the placental vascular bed that consisting of fibrin deposit, thrombosis and infarct in the women pre-eclampsia placenta who have dyslipidemia [15-16]. It is therefore suspected that there is a similar pathogenesis between pre-eclampsia and atherosclerosis associated with the condition of dyslipidemia through an unknown mechanism. Theoretically, it is should not occur dyslipidemia due to lipid homeostasis that regulated by transcription factors which known as sterol regulatory element binding protein (SREBP).

This paper examines the mechanisms of pre-eclampsia pathogenesis through placentation examination that associated with oxidative stress processes in the placenta. It is suspected that oxidative stress in the placenta is due to the high expression of SREBP-2 which may explain the condition of dyslipidemia in pre-eclampsia that ultimately causes placental atheros and placental hypoxia, as well as low 2-ME expression results in failure of spiral arterial remodeling result in the high expression of F2IsoP that causes endothelial dysfunction and the appearance of pre-eclampsia syndrome.

**Study Design**

This was an unpaired case-control. The case is a labor with a singleton live pregnancy with pre-eclampsia. Control is a labor with a singleton live pregnancy without pre-eclampsia. Risk factors are F2-Isoprostan (F2-IsoP), Sterol Regulatory Element Binding Protein-2 (SREBP-2) and 2-Mehoxyestradiol (2-ME).
The location of study is in Maternity Room at Emergency Department of Sanglah Hospital Denpasar, Pathobiology Laboratory of Faculty of Veterinary Medicine Udayana University and Histology Laboratory of Faculty of Medicine Udayana University. Study starts from April 2015 to September 2015.

Patients who meet the inclusion and exclusion criteria and had signed an informed consent, conducted placental tissue sampling and examination Immunohistochemistry and Elisa. The data is processed using SPSS. Data analysis in this study includes several tests as follows: normality test for the age, gestational age and parity data with the Shapiro-Wilk test, homogeneity test data to know the data variance using Levene Test, calculating F2isoP expression odds ratio, SREBP-2 and 2-ME using the Chi-square, and measuring the contribution F2isoP, SREBP-2 and 2-ME using path analysis test.

Results
A case control study has been carried out on 62 pregnant women in the age range 20 weeks to 40 weeks in the Delivery Room Emergency Room (ER) General Hospital Sanglah since June 2015 to September 2015. Samples were taken by consecutive sampling. 62 pregnant women, 31 pregnant women who deliver with diagnosis of preeclampsia were recruited as a case group, and 31 pregnant women who deliver without preeclampsia were recruited as a control group. From each group, the placenta tissue was taken by using a 3 cm x 3 cm scalpel for examination F2-Isoprostane (F2-Isop) expression, Sterol Regulatory Element Binding Protein (SREBP-2) and 2- Methoxyestradiol (ME).

The tissue is put into a pot containing 10% buffered formalin and labeled sample number, then stored in the refrigerator (freezer) at -70 °C in Patobiology Laboratory of Veterinary Medicine Faculty of Veterinary Medicine Udayana University.

Examination of F2-Isoprostane (F2-Isop) and Sterol Regulatory Element Binding Protein (SREBP-2) using immunohistochemical method, and 2-Methoxyestradiol (2-ME) examination using ELISA method.

This study has been approved for ethical eligibility from the Research Ethics Committee of Medical Faculty of Udayana University/General Hospital Sanglah Denpasar dated July 7, 2015, number: 1360 / UN.14.2 / Lithbang / 2015.

Normality data test against the data of maternal age, gestational age, and parity, tested using the Shapiro-Wilk Test. The results show normal distributed data (p> 0.05). Homogeneity of maternal age data, Age of pregnancy and parity, tested using Levene test.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Case Group (n=31)</th>
<th>Control Group (n=31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean 27.48, SD 6.79</td>
<td>Mean 26.84, SD 6.58</td>
<td>0.705</td>
</tr>
<tr>
<td>Parity</td>
<td>Mean 0.58, SD 0.81</td>
<td>Mean 0.94, SD 0.80</td>
<td>0.106</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>Mean 37.35, SD 1.68</td>
<td>Mean 37.68, SD 1.28</td>
<td>0.399</td>
</tr>
</tbody>
</table>

The results show homogeneous data (p> 0.05). In this case-control study, we conduct independent t-test for age, parity and gestational age. As shown in the table 3, the variables maternal age, parity and gestational age p value for each risk factor is> 0.05, which states that statistically there was no significant difference between the two groups of variables.

To determine the role of variables against the risk of pre-eclampsia Chi-Square test was used. Table 4 above shows that high F2-Isoprostane expression is a risk factor for pre-eclampsia of 4.44 times (OR = 4.44, CI 1.53 - 12.94; p = 0.005) compared with low F2-Isoprostane. High SREBP-2 expression is a risk factor for pre-eclampsia of 8.19 times (OR = 8, 19; CI 23.11 - 29.073; p = 0.000) compared with low SREBP-2 expression. We also found that low 2-ME level is a 5-fold risk factor for pre-eclampsia (OR = 5.23, CI95% = 1.75-15.55; p = 0.002) compared with a high 2-ME expression.

Table 1: Distribution of maternal age, parity, and gestational age characteristics in both groups
Table 2: Risk of Preeclampsia on High F2-Isoprostan Expression

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>CI 95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2-Isoprostan (Cut off score 3, 6811%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>22</td>
<td>11</td>
<td>4.44</td>
<td>1.53-12.94</td>
<td>0.005</td>
</tr>
<tr>
<td>Low</td>
<td>9</td>
<td>20</td>
<td></td>
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</tbody>
</table>

Table 3: Risk of Pre-eclampsia on High SREBP expression

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>CI 95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SREBP-2 (Cut off score 200%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>17</td>
<td>4</td>
<td>8.19</td>
<td>2.311-29.073</td>
<td>0.001</td>
</tr>
<tr>
<td>Low</td>
<td>14</td>
<td>27</td>
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</table>

Table 4: The risk of pre-eclampsia at low 2-ME levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>CI 95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-ME (Cut off 3.515 pg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>20</td>
<td>8</td>
<td>5.23</td>
<td>1.75-15.55</td>
<td>0.002</td>
</tr>
<tr>
<td>High</td>
<td>11</td>
<td>23</td>
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Discussion

Until now the influence of maternal age on the occurrence of pre-eclampsia is controversial. In developed countries where an increase in the age of pregnant women over 35 years reported a relationship between the advanced maternal age or over 35 years with the occurrence of pregnancy complications such as abortion, fetal death, gestational hypertension and pre-eclampsia.

Many factors are not known how the relationship between maternal age and the incidence of pre-eclampsia. Therefore, pre-eclampsia events related to maternal age are controversial. This is supported by Gold, who mentions that there is still controversy about the relationship between maternal age and the incidence of pre-eclampsia. This is in accordance with the results of this study where obtained the mean of gestational age in the case group was 37.35 weeks and 37.68 weeks in the control group. From the results of independent t-test in both parameters were not found significant differences between the two groups (p = 0.705), so it was concluded that the maternal age had no effect on the incidence of pre-eclampsia.

The gestational age in pathophysiological have an influence on the risk of pre-eclampsia, pre-eclampsia can appear at the gestational age less than 34 weeks, called early onset preeclampsia and gestational age over 34 weeks called late onset preeclampsia. This is related to the pathogenesis of preeclampsia where preeclampsia occurs due to the failure of trophoblast invasion into the spiral artery occurring at the beginning of the first trimester at 16-18 weeks gestation, so that clinical manifestations will appear at gestational age more than 20 weeks. Most pre-eclampsia occur at gestational age > 34 weeks (2.7%-88%) of all pre-eclampsia cases, whereas only a few case occurs at <34 weeks (0.38% - 12%) gestational age, it is related to the extent of vascular lesions in the placental villi [17].

It was reported by Yazdani and colleagues that they found no significant differences between gestational age <34 weeks with gestational age > 35 weeks in the presence of pre-eclampsia syndrome (p = 0.05) [18]. This is in accordance with the results of this study, where obtained the mean of gestational age in the case group was 37.35 weeks and 37.68 weeks in the control group. After the independent t-test, the two groups showed no significant difference (p = 0.399). Therefore, it can be concluded that differences in gestational age have no effect on the occurrence of pre-eclampsia.

Nulipara is also one of the risk factors for pre-eclampsia. In a cohort study conducted by Xun Li in Liu Yang found that the risk for pre-eclampsia in nulliparas in the study population was 1.1 (0.73-1.66) but this was not statistically significant (p = 0.657) [19]. Also reported by Yazdani et al., they found no significant difference between primiparas and multiparas in the appearance of pre-eclampsia syndrome (p = 0.06) [18]. This is in accordance with the results of this study where the mean of parity of 0.58 in cases and 0.81 in the controls group with a mean of 0.94 and the difference of parity in both
groups was not significantly different ( \( p = 0.106 \)). It can be concluded that parity in these two study groups had no effect on the incidence of pre-eclampsia.

**F2-IsoProstane**

F2-IsoP is a results peroxidation metabolite of arachidonic acid by free radicals, through free radical-calatyzed mechanism and does not depend on the role of cyclooxygenase enzymes. F2-IsoP has a fairly stable chemical structure, formed at the site of an attack of free radicals, then immediately circulates in the blood and excreted through urine [20]. F2-IsoP has four isomers, namely 5, 8, 12, and 15 series. Series 8 or 8-isoprostone, is the most widely generated F2-IsoP isomer and is the most studied F2-IsoP. F2-IsoP has been found almost in all biological fluids, including plasma / serum, urine, joint fluid, bronchoalveolar fluid, bile, lymph fluids, microdialysis fluids from various organs, amniotic fluid, pericardial fluid and seminal fluid. For the purposes of sampling study, plasma and urine samples are the most commonly used because it is most readily available and least invasive [10].

Available current data also show that measurements of F2-IsoP levels from plasma, serum, and urine provide accurate and precise results from the oxidative stress index, but their value is still affected by plasma volume and renal excretion capacity [10-21-8-9]. Several studies using F2-IsoP to examine the association between increased lipid peroxidation and pre-eclampsia found that higher F2-IsoP levels in preeclampsia patients than non-preeclampsia patients [22]. As well as, that plasma 8-isoprostone levels were higher in the group of preeclampsia compared with normal pregnancy (354 ± 218 ± 232 vs. 149 pg / mL, \( p = 0.02 \)) [15].

The process of vascular remodeling in the spiral artery is important to ensure adequate blood supply from the mother to the placenta. The spiral artery remodeling occurs at gestational age less than 14 weeks to 18-20 weeks, with the end result of widening spiral artery lumen resulting in an increase of supply blood to the placenta [23]. The failure of the spiral artery remodeling process in pre-eclampsia leads to oxidative stress conditions caused by imbalanced free radicals and endogenous anti-free radicals, which has been shown that endogenous antioxidant activity was decreases. As a result of the increase of free radicals, particularly free radical anion superoxides result in damage to cells. Clinical manifestations of such damage occur at gestational age greater than 20 weeks [24-22-25-26-27-28].

Evidence of placental oxidative stress conditions in this study can be seen from the high expression of F2-IsoP in placenta pregnant women with pre-eclampsia compared with no pre-eclampsia.

Immunohistochemical results of F2-IsoP from the placenta have an advantage because they reflect the event of oxidative stress in the placenta. This is supported by the statement of Bilodeou, which states that the source of F2-IsoP in pregnant women can be from two sources, from the placenta and mother since the beginning of pregnancy. Therefore, to know the events of oxidative stress in pre-eclampsia it is better done on the placenta as a source of the emergence of clinical syndrome pre-eclampsia. The measurements of F2-IsoP from plasma and urine have greed because it is influenced by the mother's systemic condition [29].

When compared with previous studies that studied F2-IsoP levels in pre-eclampsia and normal pregnancies taken from blood samples, there found of 21 from 27 cases of pre-eclampsia (77%) [5]. It is higher than this study where F2-IsoP expression studied directly from the placenta by immunohistochemical technique found that F2-IsoP expression of 22 from 31 placenta cases of pre-eclampsia or as much as 70%. This may be due to the F2-IsoP examination of the plasma affected by the mother's condition.

The difference between previous study and this study is F2-IsoP expression examination in such study using ELISA method, while this studies using immunohistochemical method, and different population characteristics. It can be concluded that the expression of F2-IsoP in pre-eclampsia is sourced from the placenta and is not affected by differences in population characteristics and mode of examination. The high role of F2-isop in the emergence of pre-eclampsia clinical syndrome is through increased proliferation of smooth muscle cells, endothelial cell stimulation, platelet...
aggregation modulation and vasoconstriction in accordance with study results on the isoprostan role in in vitro angiogenesis reported by [30]. Although it is practically and ethically difficult to carry out F-isoP examinations on the placenta to predict the occurrence of preeclampsia, academically the results of this study may serve as a basis for knowledge to further investigate the association of F2isoP levels in the placenta and in blood.

**Srebp-2 (Sterol Regulatory Element Binding Protein-2)**

The growing cells require cholesterol and fatty acids according to the level of cell growth requirements. In certain tissues such as liver and endocrine glands, cholesterol biosynthesis pathways are associated with bile acid synthesis and steroid hormones. The synthesis of fatty acids and triglycerides is referred to as lipogenesis, which is the body's energy supply system in the liver and fat tissue. Instead, syntheses of cholesterol are tightly controlled by a feedback system to control cholesterol levels in plasma. In addition controlled by such system, the control flow of the synthesis of cholesterol, especially at the level of transcription at the cellular level is a transcription factor called Sterol Regulatory Element Binding Protein or SREBP [31].

SREBP has 3 isoforms, namely SREBP 1-a, SREB, 1-c and SREBP-2. SREB1a and SREB1c are derived from the same gene, the single gene on chromosome 17 p11.2, whereas SREBP-2 comes from a different gene, the gene on chromosome 22 q13. SREBP 1-a and SREBP-1c are weaker than SREBP-2 although they can activate genes that induce the formation of lipogenic enzymes, where SREBP-2 is a major transcription factor regulating cholesterol biosynthesis [32]. SREBP-2 directly activates the 30 genes that produce enzymes for the synthesis of cholesterol, fatty acids, triglycerides and phospholipids, namely HMG Co-A synthase[31]. In a study conducted by Shimano in 2001 it was found that SREBP1-c was less sensitive than SREBP-2 in terms of inducing SRE in the cell nucleus. This difference is thought to be caused by the presence of the phenomenon of "lipid-sensing mechanism of SCAP", where SREBP-2 works on the level of cleavage that produces the enzyme responsible for the biosynthesis of cholesterol, while SREBP-1 works at the level of the formation of enzymes lipogenic besides HMG Co-A [33].

From the above description of SREBP-2 expression measurement in this study, based on consideration of difference of sensitivity and selectivity between transcription factor SREBP1-c and SREBP-2, and SREBP-2 measurement is done from consideration of its role in cholesterol synthesis. The association between SREBP-2 expressions in the pathogenesis of pre-eclampsia can be explained by the increase of lipid fractions or dyslipemia in the case of preeclampsia. This is supported by various studies, includes as stated by various studies [34-35-13].

It is also supported by study conducted at Sanglah Hospital that also found a significant difference in higher LDL/HDL ratio in pre-eclampsia compared with normal pregnancy. This suggests that LDL and HDL play an important role in the occurrence of pre-eclampsia. With higher LDL levels and lower HDL levels indicated by increased LDL and HDL ratios leads to the occurrence of pre-eclampsia by causing endothelial dysfunction resulting in vasoconstriction and hypertension [36]. It was also stated that there was a pregnancy with a gestational age more than 42 weeks. The incidence of placental insufficiency was 10-12%, which influenced the synthesis of LDL cholesterol through the mechanism of decreased lipolysis and increased VLDL synthesis [37].

Meanwhile, the pathologic lesions that seen in the placenta of patients with pre-eclampsia are arteriopathic necrosis that consisting of fibrinoid necrosis, accumulation of foam cells or lipid-laden macrophages in the decidua, fibroblast proliferation and perivascular infiltrate. These lesions are also known as acute atherosis [34]. In pregnancy with dyslipidemia, acute atherosis occurs only in spiral artery. There are about 30-60 spiral artery branches that provide blood supply to the inter vilous space. Large blood flow is possible because of the process of remodeling the spiral artery of the artery that has a small lumen to the artery with a large lumen. Inadequate remodeling process results in uterine placental circulatory dysfunction resulting from oxidative stress and endoplasmic reticular stress resulting in trophoblast surface architecture damage [34]. On the other hand, the non-destructive part causes vasoconstriction and intermittent
blood flow which eventually leads to ischemic-reperfusion injury with the end result of oxidative stress conditions in the placenta[34]. In pre-eclampsia, acute atherosclerosis is reported to be 20-40%, resulting in reduced spiral artery caliber and consequent decline in utero-placental circulation[34], a statement denied previous theories by and Meekins Et al. Suggesting that acute atherosclerosis is a pathologic spiral artery that occurring also in normal pregnancy as a response to the inflammatory stress of the pregnancy process, because only 8% acute atherosclerosis in normal pregnancy and does not cause classic symptoms of pre-eclampsia 34-38).

Staff,[34] proposed a theory of the acute atherosclerosis pathway that acute atherosclerosis is caused by an increase in inflammatory stress in the decidua as a result of oxidative stress, supported by genetic factors, essential hypertension, diabetes, metabolic syndrome and obesity. It can also be said that acute atherosclerosis occurs from the presence of fetal-maternal intolerance, resulting in poor placentation resulting in oxidative stress and the occurrence of pro-atherogenic lipid accumulation leading to acute atherosclerosis[34].The term "acute" in atherosclerosis placenta showed that the incidence of atherosclerosis in spiral arteries occur in a short period (during pregnancy), which is different from the classic atherosclerosis in the coronary arteries. The acute atherosclerosis occurs in the spiral artery in the decidua and in the miometrial region characterized by foam cells containing subendothelial lipid, fibrinoid necrosis, and perivascular lymphocytic infiltration. The discovery of elevated levels of TNF-α in foam cells reinforces the alleged role of inflammation in the process of formation of atherosclerosis. The combination of inflammatory stress with dyslipidemia leads to atherosclerosis resulting in a placenta having hypoxia[38].

It has been demonstrated that SREBP-2 activates genes that produce enzymes for the cholesterol synthesis, fatty acids, triglycerides and phospholipids so may explained the incidence of dyslipidemia in pre-eclampsia[39]. Although it is practically and ethically difficult to perform a placental SREBP-2 examination to predict the occurrence of preeclampsia, academically this study proves an increase in placental SREBP-2 expression in pre-eclampsia that may explain the occurrence of dyslipidemia, so the authors suggest for checking the lipid profile in any risk pre-eclampsia pregnancy.

2-Me (2-Methoxyestradiol)

During pregnancy steroid hormones are synthesized in large part by placenta, in small part by maternal and fetus. Both estrogens are necessary for the growth of the reproductive organs, the preparation of labor and other metabolic changes during pregnancy and the puerperium. In carrying out the function, placenta as a producer of estrogen and progesterone requires precursors, namely cholesterol [40-41].

In the uteroplacental unit estrogen will be converted by cytochrome P450 (CYP450) into several hydroxylated metabolites determined by the position of hydroxylation ie 2-hydroxyestrone, 4-hydroxyestrone, 16-α-hydroxyestrone, 2-hydroxyestradiol and 4-hydroxyestradiol [42]. The hydroxylated estrogen will undergo methylation with the help of the cathecol-o-methyltransferase (COMT) enzyme which present in the placenta will be converted into several metabolites ie 2-methoxyestrone, 3-methoxyestrone, 4-methoxyestrone, 2-methoxyestradiol and 4-methoxyestradiol [43]. Primary estrogens do not fully play a role in pregnancy because there is evidence that estrogen metabolites play a greater role in cardiovascular adaptation during pregnancy. The investigator attention on the role of estrogen metabolites especially 2-methoxyestradiol (2-ME) in terms of the pathogenesis of pre-eclampsia is still small and shows different results.

2-ME is a stable estrogen metabolite and has a direct effect on the vascular system through inhibition of the growth of vascular smooth muscle cells and prevents the onset of atherosclerosis and vascular dysfunction. Serum levels of 2 ME in pregnancy increased from 2-15 nmol /L starting at 11-16 weeks of gestational age and peaking at 37 weeks of gestational age with serum levels of 18-96 nmol / [44]. The 2-ME study over the last 10 years has found evidence of a 2-ME working mechanism in tumor prolongation and antiangiogenic activity. It is suspected to play a role in the pathogenesis of preeclampsia through the mechanism of uteroplacental circulatory vascular growth and inhibition of angiogenic factors and factors inducing tissue hypoxia [42-45].
In 2008, Kanasaki first reported COMT and 2-ME deficiency in preeclampsia with studies in COMT-deficient mice showing a phenotype similar to pre-eclampsia [43]. In subsequent studies, COMT deficiency increases Hypoxia Inducible Factor α (HIF-α), which causes placental hypoxic [42-46-47].

HIF-α, is the main regulator of oxygen homeostasis. Accumulation and increased HIF-α will suppress the expression of the angiogenic factor such as vascular endothelial growth factor (VEGF) and increase anti angiogenic factor Soluble fms like tyrosine kinase (sFlt-1), causing hypoxia and vascular defects in the placenta [43]. The effect of this suppression sFlt1 due to the 2-ME decrease which associated with the increase of HIF-α, was also reported in Partegal M study, where a negative correlation between 2-ME plasma levels decreased and plasma levels sFlt1 [48]. On the other hand there are differences in the results of the study content of 2-ME, Seoul reported that the level of 2-ME in the plasma of pregnant women with late onset of pre-eclampsia is higher compared to normal pregnancy, this is caused by differences in the study sample, which is the ratio between the patient’s pre-eclampsia with non pre-eclampsia and the method of examining the study sample and presumably high 2-ME levels are caused by compensatory mechanisms to protect the endothelium from damage by inhibiting HIF-α activity [46].

The 2-ME role in placental vascularization growth is evidenced by increasing levels of 2-ME throughout gestational age and has begun since first trimester pregnancy, so that if levels decrease it may precipitate pre-eclampsia [47]. The relationship between deficiency of 2-ME to the occurrence of early onset pre-eclampsia has been reported by Zhang et al, by screening plasma concentrations of 2-ME, estrogen (E2), sFmsLt-1 and Nitric Oxide (NO) in 28 patients with pre-eclampsia and 20 pregnant patients without pre-eclampsia at gestational age between 24-32 weeks. The results are lower plasma 2-ME and plasma sFmsLt1 levels were higher in pre-eclampsia patients compared with 2-ME levels in patients without pre-eclampsia (p <0.003). There was no significant difference in estrogen and ANY levels in patients with pre-eclampsia and patients without pre-eclampsia. It is clear that estrogen is not much role in the pathogenesis of pre-eclampsia compared with estrogen metabolites, 2-ME, especially early onset pre-eclampsia [45].

Although the hypoxic condition of the placenta is a necessary condition in early pregnancy for a complete trophoblast invasion, the hypoxic condition is not the only condition that ensures the ongoing process of trophoblast invasion, Lee, stated that in placenta which made hypoxic by cell culture experiments under hypoxic conditions (O₂ 2.5%), it was found an increased expression of 2-ME is a 17-8 estradiol metabolites synthesized by the enzyme COMT. Vice versa, there are no 2-ME on the expression of normoxic placental condition [47].

In relation to the action mechanism of 2-ME in the pathogenesis of pre-eclampsia, it is stated that 2-ME works to maintain placental homeostasis by regulating trophoblast invasion process along first trimester pregnancy. The low concentration of 2-ME and the placenta in hypoxic conditions cause trophoblast cells remain in a non-invasive phenotype. In this study, a low level of 2-ME is a risk factor preclampsia 5-fold higher than the high level of 2-ME (OR = 5.23; 95% CI = 1.75 to 15.55; p = 0.002).

These results are consistent with the results of study conducted by Sepulveda, 2012 were found that the plasma levels of 2-ME as measured at the gestational age of 11 weeks-14 weeks lower in pregnant women who develop into pre-eclampsia compared with pregnant women who did not develop into pre-eclampsia (1.9 ± 2 pg/dl vs. 61.7 ± 27 pg/dl, p <0.05) [47]. The results from Lee in 2010 also found that suppression of HIF-α and TGF-83 in high serum levels of 2-ME with oxygen pressure conditions over 18 mmHg, whereas in hypoxic conditions, O2 pressure of less than 2.5%, 2-ME levels become low and an increase in HIF-α and TGF-83. Under hypoxic conditions with a low level of 2-ME, it is result in cytotrophoblast are in the phenotype that is not invasive, causing the failure of the invasion trophoblast into the decidua maternal and uterus, and this condition is highly dependent on the gradient of the concentration of oxygen between the placenta, decidua and uterus [44].
Based on these results and the above descriptions, it can be concluded that 2-ME play a role in the failure of the trophoblast ekstravilus invasion process into the decidua and uterus through the stimulation of the expression of HIF1-α and TGF-B3 that cause changes trophoblast into a non-invasive form and result in placental hypoxia. This can explain the role of 2-ME on the mechanism of occurrence of pre-eclampsia.

When seen from the above description, it can be explained that pre-eclampsia is a pathological event that involving placental oxidative stress and subsequently induces an increased expression of SREBP-2 result in an F2-IsoP, and simultaneously decreased the expression of 2-ME led to placental hypoxia. F2-IsoP role, SREBP-2 is highly evident by the high expression of two proteins in the placenta and the low expression of 2-ME as compared to expression in normal pregnancy without preeclampsia. The difference are statistically significant, and is a risk factor for pre-eclampsia, while other mechanisms are suspected cause of pre-eclampsia has been removed through the design and analysis.

Role of F2-IsoP, SREBP-2 and 2-ME Expression on the Pre-eclampsia Mechanism

In the conventional theories of the pre-eclampsia pathogenesis mechanisms has been known that pre-eclampsia occurs as a result of events that began with the failure of spiral artery remodeling that leads to hypoxia-reperfusion injury of the placenta resulting in oxidative stress and endothelial dysfunction of the placenta resulting in the emergence of clinical a pre-eclampsia syndrome which is hypertension and proteinuria. While other factors such as parity, maternal age factor are also said to affect the occurrence of pre-eclampsia. This condition confirms that pre-eclampsia is the result of the interaction of various factors of risk and spiral artery remodeling failure that induced endothelial dysfunction.

This study found that parity, maternal age and gestational age had no effect on the pre-eclampsia mechanism. The pre-eclampsia mechanism begins with a significant increase in the expression of placental SREBP-2 evidenced by the difference in the high expression of SREBP-2 placenta in case (pre-eclampsia) compared with expression of SREBP-2 placenta in control (without pre-eclampsia) and found that the high expression of SREBP-2 in the placenta of pre-eclampsia is a risk factor for pre-eclampsia by 8.19 times with OR 8.19; CI 2.31 to 29.073 compared with low expression of SREBP-2 (p = 0.005). The high SREBP-2 in pre-eclampsia may explain the occurrence of dyslipidemia, particularly an increase in LDL and oxidized LDL that contributes to the formation of foam cells in the decidua and induces atherosclerosis. Such conditions resulted in inadequate placentation, causing hypoxia-reperfusion and subsequently cause the placenta under conditions of oxidative stress as evidenced by the difference in high expression of placental F2isoP case (pre-eclampsia) compared with expression F2isoP in controls (without pre-eclampsia) and found that in high F2isoP expression in the pre-eclampsia placenta is a risk factor for pre-eclampsia by 4.44 times with OR 4.44; CI 1.53 to 12.94, compared with a low F2isoP expression (p = 0.001).

F2-IsoP directly cause endothelial activation, proliferation of smooth muscle cells of blood vessels and platelet aggregation. This condition is simultaneously exacerbated by the low expression of 2-ME in placental case (pre-eclampsia) compared to the expression of 2-ME in placenta control (without pre-eclampsia) and found that the low expression of 2-ME in placenta of pre-eclampsia are risk factors for pre-eclampsia by 5.23 times with OR 5.23; CI 1.75 to 15.55 compared with high 2-ME expression (p = 0.002). With the low expression of 2-ME will induce an increase in HIF-α and causes the placenta to hypoxia because cytotrophoblast become non-invasive.

According to path analysis, this study found also their contribution of SREBP-2 is larger (46%) followed by F2isoP (32%) and 2-ME (31%) in the pre-eclampsia pathogenesis mechanisms. This confirms that placental hypoxia events are not always preceded by the failure of the spiral artery remodeling, but can be caused by the decreased blood flow to the placenta as a result of their dyslipidemia induced by high expression of SREBP-2, followed by the low expression of 2-ME. Both will lead to high expression of F2-IsoP in the placenta which will directly lead to the emergence of symptoms or syndrome of pre-eclampsia.
Conclusion

Based on the results and discussion, we conclude that high expression of placenta F2-isoprostane (F2-Isop) and placenta Sterol Regulatory Element Binding Protein-2 (SREBP-2), and also low expression placenta 2-Methoxyestradiol (2-ME) is a greater risk factor for the occurrence of pre-eclampsia. We recommend further study on the relationship between increased expressions of F2-Isop and SREBP-2 and decrease in 2-ME with dyslipidemia in pre-eclampsia that associated with the pre-eclampsia pathogenesis Laboratory investigation on plasma F2-isoprostane levels, Sterol Regulatory Element Binding Protein-2 and 2-Methoxyestradiol semiquantitatively can be considered. [49-81]

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