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Low Expression Of 2-methoxyestradiol (2-me) On Placenta Tissue As A Risk Factor Of Pre-eclampsia

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Pre-eclampsia (PE) still a problem of Maternal Fetal Medicine service 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 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 low 2-ME expression in the placenta as a risk factors for PE and a 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 mother with PE and control groups consisted of 31 non-PE mother. The study material is a placental tissue. Examination of 2-ME expression 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 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.

Keywords: Pre-eclampsia, 2-Methoxyestradiol.

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 (Gupta *et al*., 2005; Powe *et al*., 2011; Cassandra, 2014). 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% (Oka and Surya, 2005),

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in 2006 was 6.06% (Oka and Surya, 2005) and in 2013 was 9.23% (Lidapraja *et al* ., 2013).

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 (Cindrova, 2009).

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 (Toescu *et al*., 2002). Lipid peroxidation is thought to play an important role in causing endothelial function disorders and the incidence of clinical symptoms of pre-eclampsia (Hung and Bruton, 2006; Borekci *et al*., 2009; Gupta *et al*., 2009).

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 (Jayante and Saha, 2006; Dalle-Donne *et al*., 2006; Aziz *et al*., 2007; Borecki *et al*. 2009). However, the opposite is stated by Bar *et al*., in 2002 that the increase in lipoprotein was not proven to be used as a predictor of pre-eclampsia (Bar *et al*., 2002). On the other hand experts say there is an analogy between atherosclerotic vascular in patients with dyslipidemia with atherosis on the placental vascular bed that consisting of fibrin deposit, thrombosis and infarct in the women pre-eclampsia placenta who have dyslipidemia (Harsem *et al*., 2007; Isezuo and Ekele 2008). It is therefore suspected that there is a similar pathogenesis between preeclampsia and atherosclerosis associated with the condition of dyslipidemia through an unknown mechanism.

This paper examines the mechanisms of pre-eclampsia pathogenesis through placental examination that associated with oxidative stress processes in the placenta. It is suspected that oxidative stress in the placenta is due to the low 2-ME expression results in failure of spiral arterial remodeling 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 factor is 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, calculation 2-ME using the Chi-square.

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 pre-eclampsia 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 2- Methoxy estradiol (ME). The tissue is put into a pot containing 10% buffered formalin and labeled sample number, then stored in the refrigerator *(freezer)* at -70 ^{0}C in Patobiologi Laboratory of Veterinary Medicine Faculty of Veterinary Medicine Udayana University. Examination of 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 / Litbang / 2015.

Normality data test against the data of maternal age, gestational age, and parity, 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.

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 1, 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 2 below shows 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.

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 the maternal age was found in case group 27, 48 years and 26, 84 years 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.

Table 1. Distribution of maternal age, parity, and gestational age characteristics in both groups

Risk factor	Case Group (n=31)		Control Group (n=31)		P
	Mean	SD	Mean	SD	
Age (years)	27,48	6,79	26,84	6,58	0, 705
Parity	0,58	0,81	0,94	0,89	0,106
Gestational age (weeks)	37,35	1,68	37,68	1,28	0,399

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

		Group		OR	CI 95%	P
		Case	Control			
2-ME(Cut off 3,515 pg/dl)	Low	20	8	5,23	1,75-15,55	0,002
	High	11	23			

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 pre-eclampsia occurs due to the failure of tropoblast 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 (Chaiworapongsa *et al*., 2014).

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) (Yazdani *et al*., 2015). 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) (Li *et al*., 2016). 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) (Yazdani *et al*., 2015). 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.

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 (Hill *et al*., 2002; Hadisaputro, 2008).

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 ±-hydroxyestrone, 2-hydroxyestradiol and 4-hydroxyestradiol (Jobe *et al*. , 2013). 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-methoxyestrdiol (Kanasaki, 2009). 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-methoxystradiol (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 / L (Lee *et al*. 2010). 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 (Jobe *et al*., 2013; Zhang *et al*., 2014).

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 (Kanasaki, 2008). In subsequent studies, COMT deficiency increases Hypoxia Inducible Factor ± (HIF-±),

which causes placental hypoxic (Jobe *et al*., 2013; Seoul *et al*., 2013; Sepulveda *et al*., 2013)

HIF-±, is the main regulator of oxygen homeostasis. Accumulation and increased HIF-± 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 (Kanasaki, 2008). The effect of this suppression sFlt1 due to the 2-ME decrease which associated with the increase of HIF-±, was also reported in Partegal M study, where a negative correlation between 2-ME plasma levels decreased and plasma levels sFlt1 (Partegal *et al*., 2014). 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-± activity (Seoul *et al*., 2014).

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 (Sepulveda *et al*., 2013). 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 NO 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 (Zhang *et al*., 2014).

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 tropoblast invasion.Lee, stated that in placenta which made hypoxic by cell culture experiments under hypoxic conditions (O_2 2.5%), it was found an increased expression of 2-ME is a 17-² estradiol metabolites synthesized by the enzyme COMT. Vice versa, there are no 2-ME on the expression of normoxic placental condition (Sepulveda *et al*., 2013).

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 (Lee *et al*., 2013). 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, $p < 0.05$) (Sepulveda *et al*., 2012). The results from Lee in 2010 also found that suppression of HIF-± and TGF-²3 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-± and TGF-²3. 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 (Lee *et al* ., 2010).

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-± and TGF-²3 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 decreases the expression of 2-ME led to placental hypoxia. 2-ME is highly evident by the low expression compared to expression in normal pregnancy without preeclampsia. The differences 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 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. These conditions 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 endothelial activation, proliferation of smooth muscle cells of blood vessels and platelet aggregation are 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-± and causes the placenta to hypoxia because cytotrophoblast become non-invasive.

CONCLUSION

Based on the results and discussion, we conclude that 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 decrease expression in 2-ME with dyslipidemia in pre-eclampsia that associated with the pre-eclampsia pathogenesis. Laboratory investigation on plasma 2-Methoxyestradiol semiquantitatively can be considered.

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