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Serum 8-isoprostane increased in pre-eclampsia

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ABSTRACT

BACKGROUND
The main causes of maternal mortality in Indonesia are pre-eclampsia, hemorrhage, and infection. Oxidative stress has a primary role in pre-eclampsia and one of its parameters is 8-isoprostane serum level. The objective of this study is to measure 8-isoprostane and to analyze the relationship between 8-isoprostane level and birth weight in pre-eclampsia.

METHODS
A cross-sectional study involving 23 pre-eclampsia and 29 normotensive pregnant women with normal or cesarean delivery at Dr. Kariadi Hospital Semarang from January to May 2011. Collected maternal blood samples were assessed for 8-Isoprostane levels by means of a specific ELISA kit. Neonatal birth weight was measured immediately after delivery by means of calibrated baby scales. Differences in 8-isoprostane levels between pre-eclampsia and normotensive women were assessed using independent t-test for normal distributed data, and the Mann-Whitney test for non-normally distributed data.

RESULTS
Mean 8-isoprostane level was significantly higher in women with pre-eclampsia than in normotensive women (62.52 ± 12.19 pg/mL vs 28.64 ± 8.81 pg/mL) ($p<0.05$). Low birth weight was twice as frequent in pre-eclampsia than in normotensives. There was no correlation between 8-isoprostane serum level and neonatal birth weight in pre-eclampsia.

CONCLUSION
The level of 8-isoprostane was higher in pre-eclampsia than in normotensives. It is recommended to conduct further studies to determine whether 8-isoprostane may be used as a predictive marker of pre-eclampsia.

Keywords: 8-Isoprostane level, birth weight, pre-eclampsia

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Kadar isoprostan-8 meningkat pada pre-eklampsia

ABSTRAK

LATAR BELAKANG

Stres oksidatif berperan penting pada pre-eklampsia, dan salah satu parameter stres oksidatif yang dapat diukur adalah isoprostan-8.Tujuan penelitian ini adalah membandingkan kadar isoprostan-8 pada pre-eklampsia dengan kehamilan normotensi dan menganalisis hubungan antara kadar isoprostan-8 dengan berat lahir bayi pada pre-eklampsia.

METODE

Penelitian belah lintang mengikutsertakan 23 ibu pre-eklampsia dan 29 ibu hamil normotensi yang melahirkan baik pervaginam/perabdominam di RSUP Dr. Kariadi Semarang selama Januari–Mei 2011. Sampel darah vena diambil pada saat persalinan kemudian diukur kadar isoprostan-8 dan berat badan bayi diukur setelah bayi lahir. Isoprostan-8 serum kemudian dianalisis korelasinya dengan berat badan lahir bayi. Analisis statistik menggunakan uji korelasi, uji beda dengan t test, dan uji Mann Whitney.

HASIL

Rerata isoprostan-8 pada ibu hamil dengan pre-eklampsia lebih tinggi dari pada kehamilan normal (62,52 ± 12,19 pg/mL dibanding 28,64 ± 8,81 pg/mL) dan bermakna secara statistik ($p<0,05$). Berat badan lahir bayi yang dilahirkan oleh ibu hamil dengan pre-eklampsia yang ≤2500 gram 2 kali lebih banyak daripada yang dilahirkan ibu normotensi. Tidak terdapat hubungan antara kadar isoprostan-8 dengan berat badan lahir bayi pada ibu dengan pre-eklampsia.

KESIMPULAN

Kadar isoprostan-8 ibu hamil dengan pre-eklampsia lebih tinggi dibanding dengan ibu hamil normotensi. Tidak terdapat hubungan antara isoprostan-8 dengan berat badan lahir bayi pada ibu dengan pre-eklampsia.

***Kata kunci**: Isoprostan-8, berat badan lahir, pre-eklampsia*

INTRODUCTION

To date pre-eclampsia and eclampsia are still one of the complications of pregnancy, labor/delivery, or the postpartum period, and is also the main cause of maternal and fetal mortality and morbidity.[1] One of the targets of "Making Pregnancy Saver" in Indonesia for the years 2001-2010 is to reduce maternal and neonatal mortality rates to 125 per 100,000 live births and 16 per 100,000 live births, respectively. Current maternal and neonatal mortality rates in Indonesia are still high, being 334 per 100,000 live births and 21 per 100,000 live births, respectively. The main causes of maternal mortality in Indonesia are pre-eclampsia, hemorrhage, and infection.[2] Worldwide, approximately 585,000 women die every year from pregnancies, 95% of them occurring in developing countries and 13% of maternal deaths is caused by hypertension during pregnancy, particularly in pre-eclampsia.[2,3]

Pre-eclampsia is a pregnancy-specific syndrome marked by increased blood pressure and proteinuria in pregnancies of more than 20 weeks.[4] Pre-eclampsia is still a disease of theories, as its pathogenesis is still not known with certainty, although all experts agree on the occurrence of vascular endothelial dysfunction and acute atherosis involving multiple organ systems.[1] Newer concepts focus on

involvement of free radicals presumably associated with endothelial dysfunction and increased lipid level due to peroxidation of the free radicals. The increase in lipid levels also increases the susceptibility of fatty acids to damage by free radicals from oxidative stress, and is marked by increased levels of 8-isoprostane.[5] 8-isoprostane is a prostaglandin-like compound produced within the tissues mainly through esterification of arachidonic acid by non-enzymatic reactions catalyzed by free radicals, and its level is also raised in pre-eclampsia. 8-isoprostane is believed to be an indicator of oxidative stress. Oxidative stress plays an important role in pre-eclampsia, in which an imbalance between pro-oxidants and antioxidants leads to endothelial dysfunction. One of the results of endothelial damage is increased vascular permeability with concomitant vascular leakage of micronutrients that are essential for the fetus.[6] Among these micronutrients are amino acids that act as fetal growth factors and affect birth weight. Various studies on the difference in isoprostane levels between women with normal pregnancies and those with pre-eclampsia have shown inconsistent results. A cross-sectional study involving normotensive pregnant subjects and those with pre-eclampsia showed total plasma 8-iso-prostane levels were not significantly elevated in pre-eclamptic women compared with control subjects during pregnancy.[7] Other studies showed different results. Women with pre-eclampsia had higher 8-isoprostane plasma levels than controls.[8] As there are no data on the relationship between serum 8-isoprostane levels and infant birth weight, it is deemed necessary to conduct a study on a possible association between serum 8-isoprostane levels and post-eclampsia birth weight. The purpose of the present study was to analyze and compare serum 8-isoprostane levels in pregnant women with pre-eclampsia and women with normal pregnancy and their association with infant birth weight.

METHODS

Research design

A cross-sectional study was undertaken between January and July 2011 at the Dr. Kariadi Hospital in Semarang.

Research subjects

The study subjects were pregnant women with pre-eclampsia and normotensive pregnant women who had deliveries in the Delivery Ward of Dr. Kariadi Hospital, met the inclusion criteria, and agreed to participate in this study. The inclusion criteria were: Normotensive women or women with pre-eclampsia, single live intra-uterine pregnancy, without chronic systemic disorders, and non-smokers. Based on a significance level of 0.05 and a correlation coefficient and power of 80%, the size of the sample was 22. Pregnancy status was differentiated into 2 categories: a normotensive pregnancy was defined as a pregnancy with normal blood pressure, while pre-eclampsia is a hypertensive condition in a pregnancy of 20 weeks accompanied by proteinuria.

Isoprostane measurement

Serum 8-isoprostane is a lipid oxidation product used to evaluate the results of oxidative stress, determined in maternal serum by means of a specific ELISA kit (CAYMAN). The serum levels were expressed in pg/mL (Bio-Rad Model 680).

Birth weight measument

Infant birth weight is the weight in grams of the unclothed baby immediately after birth, determined by means of calibrated baby scales (TANITA).

Statistical analysis

Differences in 8-isoprostane levels between pre-eclampsia and normotensive women were assessed using independent t-test for normal distributed data, and the Mann-

Table 1. Characteristics of preeclamptic and normotensive pregnant women

Characteristics	Pregnancy status		p
	Pre-eclampsia (n=23)	Normotensive (n=29)	
Age (years) ‡	29.37 ± 7.96	29.77 ± 6.48	0.71¥
Age of pregnancy (weeks) ‡	39.11 ± 1.08	39.01 ± 1.25	0.62¥
Pregnancy category			
1	12 (52.2%)	11 (37.9%)	
2 – 4	8 (34.8%)	15 (51.7%)	
≥ 5	3 (13.0%)	3 (10.4%)	0.51
Body weight (kg) ‡	68.73 ± 12.61	61.42 ± 9.10	0.03¥
Height (cm) ‡	156.82 ± 4.48	154.13 ± 6.70	0.11§
Body mass index ‡	28.19 ± 5.72	25.95 ± 3.33	0.13¥
Blood pressure (mmHg)‡			
Systolic	165.61 ± 23.51	120.73 ± 11.00	-
Diastolic	107.45 ± 13.89	78.28 ± 5.39	-
Hemoglobin concentration	12.26 ± 1.43	11.38 ± 1.39	0.06

¥Mann-Whitney test; *χ^2 test; § Unpaired t-test; ‡ Mean ± standard deviation

Whitney test for non-normally distributed data. All tests were two-tailed with statistical significance assessed at the p-value of <0.05. All computations were done with SPSS Software version 15.0.

Ethical clearance

This study was approved by the Ethics and Research Commission, Faculty of Medicine, Diponegoro University, Dr. Kariadi Central Hospital, Semarang.

RESULTS

Characteristics of normal pregnant women and preeclamptic patients are shown in Table 1. Table 1 shows that the mean age of normotensive pregnant women was slightly higher than women with severe pre-eclampsia, but according to statistical analysis this age difference was not significant (p=0.71). In Table 1 it is also apparent that mean systolic pressure in women with pre-eclampsia was over 160 mmHg, being 165.61 ± 23.51 mmHg, whereas in normotensive pregnant women it was within normal limits, being 120.73 ± 11.00 mmHg. Mean diastolic pressure in women with pre-eclampsia was 107.45 ± 13.89 mmHg, while in the group of normotensive pregnant women it was 78.28 ± 5.39 mmHg.

Characteristics of infants born from women with pre-eclampsia and from normotensive women are shown in Table 2.

Table 2. Characteristics of infants of preeclamptic and normotensive pregnant women

Characteristics	Pregnancy status		p
	Pre-ecclampsia (n=23)	Normotensive (n=29)	
Gender*			
Male	12 (52.2%)	17 (58.6%)	0.64
Female	11 (47.8%)	12 (41.4%)	
Birth weight (g) ‡	2832.60 ± 685.36	2984.11 ± 453.57	0.31

* χ^2 test; ‡ Mean ± SD

Table 3. Serum 8-isoprostane levels (pg/mL) of preeclamptic and normal pregnant women

Pregnancy status	Mean ± SD	Median	p*
Pre-eclampsia	62.52 ± 12.19	34.90	0.04
Normotensive	28.64 ± 8.81	27.90	

*Mann-Whitney test

In Table 2 it is apparent that both in women with severe pre-eclampsia and in normotensive pregnant women, the majority of babies were of male gender. However, according to statistical analysis, the difference in gender was statistically not significant (p=0.64). Similarly, birth weight of infants from pre-ecclamptic mothers was lower in comparison with infants from normotensive mothers, although statistical test results showed non-significant differences in weight distribution (p=0.31).

Serum 8-isoprostane levels of preeclamptic and normal pregnant women are shown in Table 3. Table 3 shows that mean serum 8-isoprostane level of women with pre-eclampsia was 62.52 ± 12.19, which is significantly higher than mean serum 8-isoprostane level of normotensive pregnant women of 28.64 ± 8.81 (p=0.04).

Table 4 presents a comparison of maternal serum 8-isoprostane levels by infant birth weight category. From Table 4 it is apparent that serum 8-isoprostane levels of women with pre-eclampsia whose infants had a birth weight of ≥ 2500 gram were higher compared to those with infants of < 2500 gram birth weight. On the other hand, serum 8-isoprostane levels in the group of normotensive pregnant women whose infants had a birth weight of ≥ 2500 gram were similar to those whose infants weighed <2500 gram at birth. However, in both cases the differences were not statistically significant, at p=0.5 and p=0.9, respectively.

DISCUSSION

The present study found that serum 8-isoprostane levels of women with pre-eclampsia were higher than those of normotensive pregnant women. These results are concurrent with a previous study by Mandang et al., who also found an increased expression of in the placenta of women with pre-eclampsia.[8] This indicates that oxidative stress in pre-eclampsia is not only present in placental tissues, but also systemically. Study results concomitant with the present study have also been reported by Ouyang et al.,[9] who also found increased serum 8-isoprostane levels in women with pre-eclampsia. 8-isoprostane is considered to be a better marker for oxidative stress and tissue damage due to free radicals, as compared with other oxidative stress parameters, such as malondialdehyde (MDA). In addition, 8-isoprostane also has a higher degree of correlation with proinflammatory cytokines, in comparison with other oxidative stress parameters.[9] The increased 8-isoprostane in pre-eclampsia is caused by increased phospholipase A2 activity, especially in

Table 4. Maternal serum 8-isoprostane levels by (pg/mL) infant birth weight category

Pregnancy status	Infant birth weight categories		p*
	≥ 2500 g	< 2500 g	
Pre-eclampsia	69.63 ± 14.92	42.37 ± 20.36	0.5
Normotensive	28.63 ± 9.06	28.67 ± 7.91	0.9

*Mann-Whitney test

placental tissues.[10] Moreover, leukocytes are reportedly capable of synthesizing 8-isoprostane. Incubation of leukocytes with plasma of pre-eclampsia patients yielded higher amounts of isoprostans-8 than did incubation of leukocytes with plasma of normotensive pregnant women.[11] The study of Tsukimori et al. demonstrated that leukocytes of patients with pre-eclampsia produced significantly higher amounts of free oxigen radicals than leukocytes of normotensive pregnant women.[12]

The results of the latter study and of previous studies support the hypothesis of increases in oxidative stress, both systemically and locally in the placenta, to explain the pathogenesis of pre-eclampsia. However, in contrast to the results of the present study, a previous study by Ishihara reported that there was no significant difference between serum 8-isoprostane levels of women with pre-eclampsia and normotensive pregnant women.[13] These differing results are presumably due to differences in gestational age range of the study subjects. In Ishihara's study the gestational age range was 27-40 weeks, while in the present study the age range was 37-42 weeks. The difference in gestational age presumably affects the production of 8-isoprostane, because of differences in placental developmental stage at different gestational ages. In addition, there is the possibility that 8-isoprostane synthesis is influenced by the degree of pre-eclampsia suffered by the study subjects. In the study by Ishihara, serum 8-isoprostane levels were not differentiated by degree of pre-eclampsia, in contrast with the present study, where all patients had severe pre-eclampsia. Pre-eclampsia does not only affect the mothers, but also fetal intrauterine growth of their infants. Previous studies have reported finding an increased risk of low birth weight and small-for-gestational-age birth weight in infants born from women with pre-eclampsia in comparison with those born from normal pregnant women.[13]

In this study there was no significant difference between birth weight of infants born from women with pre-eclampsia and those born from normotensive pregnant women. The proportion of low infant birth weight in the group of women with pre-eclampsia in the present study was 11%, which is lower than that found in previous studies of low birth weight and small-for-gestational-age birth weight in infants born from women with pre-eclampsia, ranging from 20-80%. The difference is caused by the fact that in those previous studies, the majority of infants were preterm, whereas in the present study all infants were fullterm. Gupta et al.[14] found no significant difference in markers of oxidative stress between patients with pre-eclampsia and normotensive pregnant women, and consider this to be due to differences in assessment methods, inadequate assessment methods, and to differences in sensitivity and specificity of the assessment methods. As mentioned previously, pre-eclampsia also affects intrauterine fetal growth. One of the parameters of intrauterine infant growth is infant birth weight, which is determined by length of pregnancy and fetal growth rate. Szymonowicz et al.[15] have reported on the occurrence of an increased risk of infants with small-for-gestational age birth weight in women with pre-eclampsia as compared with the risk in normotensive pregnant women. In addition, these studies found a proportion of 20-80% for small-for-gestational-age infants in pre-eclampsia, whereas the present study found the smaller proportion of small-for-gestational-age infants in women with pre-eclampsia to be lower, namely 11.54%. This study also found no significant difference between infant birth weight from women with pre-eclampsia and that from normotensive pregnant women. Furthermore, the results of the present study are consistent with those of the study conducted by Xiong et al.,[16] who also found no significant difference in infant birth weight from women

with pre-eclampsia and infants of gestational age of ≥ 37 weeks. A significantly lower infant birth weight in comparison with that of infants from normotensive pregnant women was only found in women with pre-eclampsia with infants of gestational age of <37 weeks. These findings may explain the results of the study by Szymonowicz et al., who found a lower birth weight in infants born from women with pre-eclampsia than that of infants born from normotensive pregnant women. The gestational age of women with pre-eclampsia and normotensive pregnant women in the study of Szymonowicz et al.[14] was 28 weeks. In contrast to the study of Szymonowicz et al., in the present study the gestational age for all subjects was 37-42 weeks. The reason for these differing results is as yet unclear. Based on the theory of placental ischemia as the underlying cause of pre-eclampsia, a decrease in uteroplacental and fetoplacental perfusion leads to a chronic reduction in fetal blood supply, resulting in impairment of intrauterine growth.[17,18]

However, other studies report that uteroplacental perfusion in women with third trimester pre-eclampsia does not differ from that in normotensive pregnant women, and may occasionally even be higher. The higher perfusion may be due to an increased cardiac output.[19] Isoprostane is one of the factors responsible for placental vasoconstriction. The present study also found a slightly negative correlation between serum 8-isoprostane levels and infant birth weight. In addition, this study also found no significant difference in maternal serum 8-isoprostane levels between the group of women with infant birth weight of ≥2500 gram and those with infant birth weight of <2500 gram. This may be caused by the fact that infant birth weight is not solely the result of placental perfusion. Sebire et al.[20] reported an association between placental histology and degree of pre-eclampsia, despite a reduction in placental perfusion, leading to the supposition of differences in compensatory mechanisms in uteroplacental perfusion between preterm and fullterm gestational age. One of the compensatory mechanisms may be an increased nitric oxide (NO) secretion resulting in improved uteroplacental circulation through vasodilation in fullterm pregnancy,[21] but this has still to be confirmed by further studies. Another possibility is that an earlier diagnosis of pre-eclampsia causes the fetus to be exposed to a longer period of hypoxia due to reduced placental perfusion from increased 8-isoprostane levels. In women with pre-eclampsia in the third trimester of pregnancy, increased isoprostane levels do not significantly affect fetal growth any more. The results of the present study shows the occurrence of increased oxidative stress in pre-eclampsia. This opens the possibility of administration of antioxidants for the management of pre-eclampsia.

Vadillo-Ortega et al.[22] reported that administration of L-arginine and antioxidant vitamins may decrease the prevalence of pre-eclampsia in high-risk populations. Based on this report, it may presumably be necessary to conduct further studies on the use of serum 8-isoprostane levels in the first trimester of pregnancy as predictor of pre-eclampsia. The availablity of accurate markers in combination with administration of antioxidants may be expected to reduce both maternal and infant mortality and morbidity from pre-eclampsia. A limitation of this study is the determination of serum 8-isoprostane levels only, without determination of urinary 8-isoprostane levels. Measurement of serum 8-isoprostane levels only does not give reflect actual 8-isoprostane levels in the body. Determination of urinary 8-isoprostane levels can provide a measure of 8-isoprostane clearance, which may be used to explain the influence of high 8-isoprostane levels on the uteroplacental circulation. Another limitation of this study is that it did not evaluate intakes of antioxidant vitamins nor blood antioxidant levels, such that it was not possible to evaluate the simultaneous effects of oxidants

and antioxidants on intrauterine fetal growth in pre-eclampsia.

CONCLUSION

Mean serum 8-isoprostane levels in pre-eclampsia are higher than those in normotensive pregnant women. It is recommended to conduct further studies to determine whether 8-isoprostane may be used as a predictive marker of preeclamsia.

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