Research Report

Effect of the Feeding of Formula Milk Enriched with Pro-Antioxidants for the Prevention of Preeclampsia; Study of High Sensitivity C-Reactive Protein (hs-CRP) and Cell-Free mRNA of Plasminogen Activator Inhibitor-1 (PAI-1) in Plasma

Pengaruh Pemberian Susu Formula diperkaya Pro-Antioksidan untuk Pencegahan Preeklampsia; Kajian terhadap High Sensitivity C-Reactive Protein (hs-CRP) dan Cell-Free mRNA Plasminogen Activator Inhibitor-1 (PAI-1) dalam Plasma

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Abstract

Objective: To evaluate the effect of the feeding of formula milk enriched with pro-antioxidants to high sensitivity C-reactive protein (hs-CRP) and cell-free mRNA of Plasminogen Activator Inhibitor-1 (PAI-1) in the first trimester, second trimester, and post labor for the prevention of preeclampsia.

Method: This is double-blinded randomized clinical trial in 8-12 weeks pregnant woman with low (below 900 μmol/l) Ferric Reducing Ability of Plasma (FRAP), as a marker of low systemic antioxidant. A total of 104 samples were collected from patients who had antenatal care in Bunda Hospital Jakarta, Budi Kemuliaan Hospital, and Dr.Cipto Mangunkusumo General Hospital from January 2007 until December 2009. From block randomization, 49 samples were allocated in the case group, and 55 samples to the control group. A formula milk enriched with pro-antioxidants was fed to the case group throughout their pregnancy, and a regular pregnancy formula milk was fed to the control group. Hs-CRP and cell-free mRNA PAI-1 test in the two groups at first trimester, second, and post labour was conducted. The incidence of preeclampsia was then compared in the two groups.

Result: Significant difference ($p<0.05$) in preeclampsia rate, 1 sample (2%) in case group and 8 samples (14.5%) in control group was found. Level of cell-free mRNA PAI-1 was significantly ($p<0.05$) lower in the case group than in control group. Difference of post labour cell-free mRNA PAI-1 level with first and second trimester was lower in case group.

Conclusion: There was significant difference in preeclampsia rate after subjects were fed with formula milk enriched by pro-antioxidants. This difference was related with different mRNA PAI-1 which started in first trimester. Lower mRNA PAI-1 level in the case group was found.

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Keywords: preeclampsia, high sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor-1 (PAI-1)

Abstrak

Tujuan: *Menilai pengaruh pemberian susu formula diperkaya pro-antioksidan terhadap kadar plasma high sensitivity C-reactive protein (hs-CRP) dan cell-free mRNA plasminogen activator inhibitor-1 (PAI-1) pada trimester pertama, kedua dan pascapersalinan sebagai usaha penurunan kejadian preeklampsia.*

Metode*: Merupakan uji klinis acak tersamar ganda pada wanita hamil trimester pertama 8-12 minggu dengan Ferric Reducing Ability of Plasma (FRAP) yang rendah (di bawah 900 μmol/l), sebagai petanda rendahnya antioksidan sistemik. Didapatkan 104 subjek penelitian yang melakukan antenatal di RS Bunda Jakarta, RS Budi Kemuliaan, dan Rumah Sakit Dr. Cipto Mangunkusumo pada periode Januari 2007 sampai Desember 2009. Setelah dilakukan randomisasi blok, didapatkan kelompok perlakuan 49 dan kontrol 55 orang. Kelompok perlakuan akan mendapat susu formula diperkaya pro-antioksidan sepanjang kehamilan, sedangkan kelompok kontrol mendapat susu hamil yang tersedia di pasaran. Kedua kelompok dilakukan pemeriksaan hs-CRP dan cell-free mRNA PAI-1 pada trimester pertama, kedua dan pascapersalinan. Luaran utama yang dipantau adalah kejadian preeklampsia pada akhir kehamilan.*

Hasil*: Terdapat perbedaan kejadian preeklampsia yang bermakna ($p<0,05$) yaitu 1 orang (2%) pada perlakuan dan 8 (14,5%) pada kelompok kontrol. Kadar hs-CRP plasma tidak didapatkan perbedaan yang signifikan antar kelompok. Kadar cell-free mRNA PAI-1 pada kelompok perlakuan secara signifikan lebih rendah dibandingkan kontrol ($p<0,05$). Delta (selisih) cell-free mRNA PAI-1 pascapersalinan dengan trimester pertama dan kedua lebih rendah pada perlakuan.*

Kesimpulan*: Terdapat perbedaan bermakna kejadian preeklampsia pascapemberian susu formula diperkaya pro-antioksidan. Perbedaan ini berhubungan dengan perbedaan kadar mRNA PAI-1 yang dimulai dari trimester pertama. Pada kelompok dengan pemberian susu diperkaya pro-antioksidan dibuktikan lebih rendah kadar mRNA PAI-1.*

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Kata kunci*: preeklampsia, high sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor-1 (PAI-1)*

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INTRODUCTION

Preeclampsia is a complication that occured in 3-7% pregnancy and still one of maternal death major cause (15-20% in developed countries) and perinatal death.[1]

The pathogenesis of preeclampsia is incompletely known. Absent transformation or incomplete remodelling of spiral arteries due to incomplete trophoblast invasion, was shown to occur during formation of the placenta in preeclampsia and fetal growth retardation.[1-3]

Placental formation begins with trophoblast invasion continued by spiral arteries remodelling. This process involving extracellular matrix degradation

which depends on proteolytic enzyme production, such as plasmin that also has a function in fibrinolytic process.[2] Plasmin production is really dependant with activator and inhibitor factors. Plasmin activator factor is tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA). One of plasmin inhibitor factor is Plasminogen activator inhibitor-1.[2,4]

Plasminogen activator inhibitor-1 (PAI-1) which is primarily produced by endothelial cell is a primary plasminogen activator inhibitor to be a plasmin.[5,6] Increase of PAI-1 in first trimester is suspected to alter placentation process (trophoblast invasion, and spiral arteries remodeling) and would lead to preeclampsia.[2]

Other mechanisms that were proved in preeclampsia are endothelial dysfunction and increased maternal inflammation response that occured in early pregnancy.[1,3] This was proven by increase of endothelial dysfunction and inflammation marker, especially C-reactive protein (CRP), in the first and second trimester in preeclampsia.[7] It was assumed that endothelial dysfunction is because of inflammation process and oxidative stress, and will also aggravate inflammation and oxidative stress condition.[8]

C-reactive protein is an acute systemic inflammation marker, produced by liver and has important role in immunology response.[5,9-11]

High sensitivity (hs) CRP is a CRP that measures using enzyme (ELISA), fluorescence, and immunoluminometric. hs-CRP measurement is more sensitive and better in showing systemic inflammation condition compare with conventional CRP measurement.[9]

There were a lot of studies that tried to prove CRP involvement as an inflammation marker and preeclampsia, but not all studies found the same result. Kumru et al found that hs-CRP level in third trimester preeclampsia patient was higher than in normal pregnancy and had a positive correlation with increase of diastolic pressure in pregnant woman. Increased CRP was not only because of systemic response inflammation in preeclampsia caused by endothelial dysfunction, but also because of higher placental apoptosis. CRP had a role in increasing trophoblast cell phagocytosis.[9] But Djurovic, et al found no relationship between inflammation and preeclampsia.[12]

In systemic endothelial dysfunction, like metabolic syndrome and atherosclerosis, hs-CRP had a relationship with PAI-1. Devaraj, et al also found that increase of CRP also increased invitro production of PAI-1.[13]

Increased production of PAI-1 was significantly found in oxidative stress at endothelial cell in preeclampsia.[4] Antioxidant treatment was proven to reduce PAI-1 production from atherosclerosis patient's endothelial cell.[14]

With involvement of oxidative stress as an important pathogenesis of preeclampsia, it was suspected that antioxidant suplement will reduce preeclampsia rate. In 2006, Ruminis et al had proved it in Indonesia.[15] But, the other studies found different result.[1]

The objective of this study was to prove that the feeding of formula milk enriched with pro-antioxidants could decrease preeclampsia rate by decreasing hs-CRP and cell-free mRNA PAI-1 mechanism starting from early pregnancy.

mRNA PAI-1 test was conducted, not directly PAI-1 protein. It was proven that mRNA being produced represented systemic genomic signal with PAI-1 production and had relationship with PAI-1 plasma level.[16]

METHODS

This study was approved by University of Indonesia Ethic Comission and all of the samples approved to participate.

This was second grade clinical trial, phase III, double blind-Randomized Clinical Trial.[17]

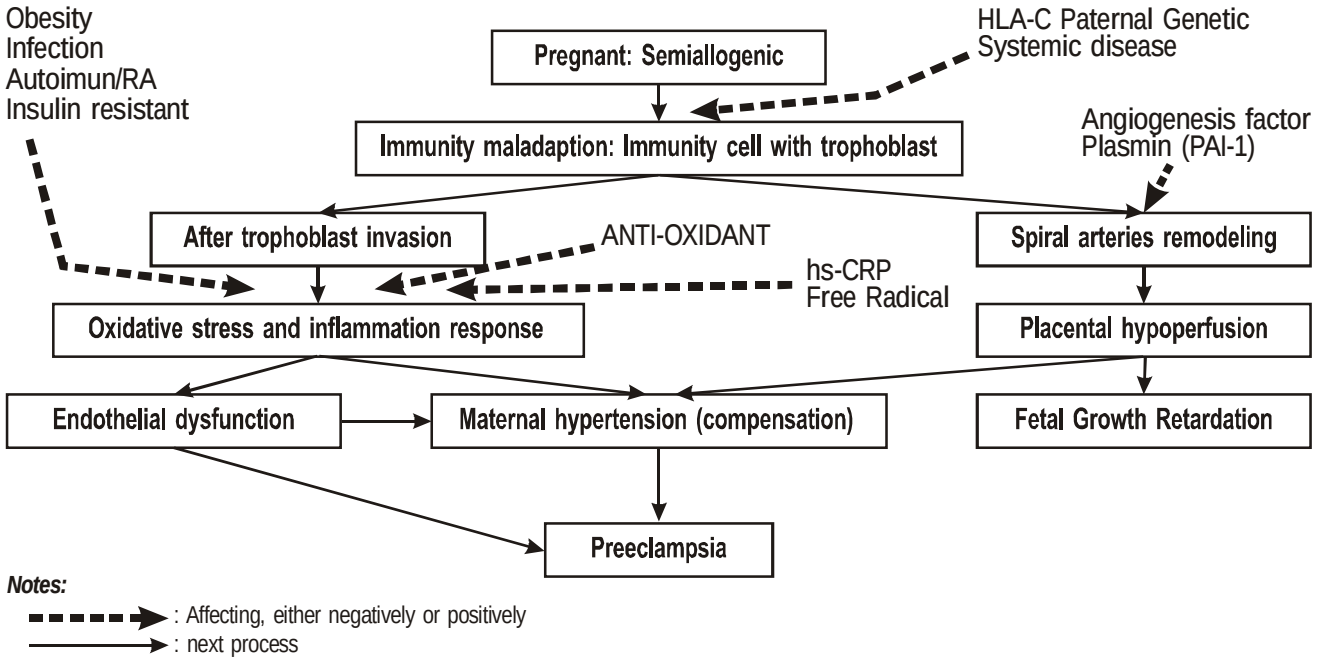

Figure 1. Preeclampsia Pathogenesis[1-2,7,10]

Samples were 8-12 weeks pregnant women who had antenatal care in Bunda Hospital Jakarta, Budi Kemuliaan Hospital, and Dr. Cipto Mangunkusumo General Hospital from January 2007 until December 2009 with FRAP level below 900 µmol/l. Consecutive sampling method was used, and 104 samples were found.

Block randomization to determine case and control group was used Case group was fed with formula milk enriched with pro-antioxidants and control group with usual milk for pregnant woman (prenagen®). Samples were fed with milk throughout their pregnancy. The milk packaging and taste were disguised, neither researchers nor patients knew.

Ingredients of formula milk feeding enriched by pro-antioxidants were vitamin A 10,000 IU, vitamin C 200 mg, vitamin E 400 IU, folic acid 400 µg, calsium 800 mg, magnesium 100 mg, selenium 100 mg, whey sistein protein 200 mg. Protein, fat and calori component were same.

hs-CRP and cell-free mRNA PAI-1 test in the first trimester (8-12 weeks), second trimester (20-24 weeks) and 2 weeks postpartum was conducted. hs-CRP test was done in Prodia laboratorium with immunolominometric method (IMMULITE® 2000). Cell-free mRNA PAI-1 test was done in Showa University, Tokyo, Japan. 5-7 cc blood samples were collected, centrifuged 1600 G about 10 minutes in 4° C. Plasma was deposited in – 20° C and posted to Japan cell-free mRNA PAI-1 test with real-time quantitave reverse transcription PCR was done Genom-equivalent was used as quantity measurement.

Samples would be dropped out if they did not complete the test.

Blood pressure, and anthropometric (body weight and height) measurement was done.

The major outcome being evaluated was preeclampsia rate. The others were pregnancy age at birth, and neonatal weight.

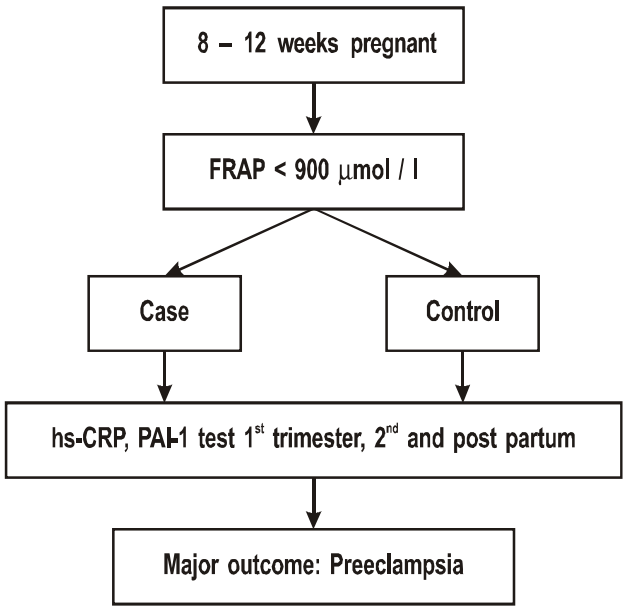

Figure 2. Research Algorithm

Definition of preeclampsia is hypertension in pregnancy (systolic pressure > 140 mmHg or diastolic > 90 mmHg) with two measurement in non dominant upper arm while sitting, in more than 20 weeks pregnancy with proteinuria (> 300 mg/day).

Pregnancy age was measured by ultrasound in first trimester. SPSS vs17 was used. If numeric data distribution was normal parametric (t-test) was used, if it was not non-parametric (Mann-Whitney) was used. Normal distribution data, was reported in mean +SD form, abnormal one was in median (minimum-maximum) form. Kolmogorov-Smirnov test we used to determine whether the numeric variable distribution was normal or not. General Linear Model-Repeated Measured test was used in hs-CRP and cell-free mRNA PAI-1 measurement serial.

RESULT

One hundred four samples who met the inclusion and exclusion criteria were found. Case group were 49 samples and control group were 55 samples. The 104 samples, were being followed until labour. There were 9 (8.6%) samples who became preeclampsia, which consisted of 1 (2%) sample from case group and 8 (14,5%) samples from control group (Table 1).

On socio-demografic data, there was no significant difference in age category. There were 5 samples with history of hypertension, 4 samples in control group, 1 sample in case group, but there was no significant difference. Body Mass Index variable in first trimester was higher in case group than in control group. (Table 1.)

Table 1. Case and control group characteristics

Characteristics	Control (n:55)	Case (n:55)	p
Age, years, mean+SD	29.7 ± 5.1	28.1 ± 5.1	0.77
Occupational status, n (%)			
Working	30	21	0.16
Not working	25	28	
Educational Status, n (%)			
Low	3	8	0.17
Moderate	24	21	
High	28	20	
Smoking condition, n (%)			
Active	14	20	0.55
Passive	1	1	
Not smoking	40	28	
History of Hypertension, n (%)			
Yes	4 (7.2)	1 (2.1)	0.22
No	51 (92.7)	48 (97.9)	
Body Mass Index, median (range)			
First Trimester	22.1 (16.2–29.9)	23.3 (16.2–42.7)	0.04*
Second Trimester	24.1 (17.3–32.5)	25.1 (18.4–44.0)	0.10
Third Trimester	26.7 (18.6–35.4)	26.7 (19.1–45.3)	0.50
Neonatal birth weight, (gram)	3200 (1500–4000)	3126 ± 451	0.57
Pregnancy age at termination, (weeks)	39 (32–43)	39 (32–41)	0.26

**) significant difference with non-parametric (Mann-Whitney) test*

There was abnormal distribution data (hs-CRP and cell-free mRNA PAI-1 plasma level) in whole samples. There was no significant difference in hs-CRP level between the two groups (Table 2).

PAI-1 level in case groups were lower than in control group, starting from first, second trimester, until postpartum (Table 3).

mRNA PAI-1 deviation in first-second trimester were not significantly different. Postpartum-first trimester deviation and postpartum-second trimester deviation was significantly different in case and control group ($p<0.05$).

Table 2. Preeclampsia outcome in each group

Preeclampsia rate	Control (n:55)	Case (n:49)	p	RRR
No preeclampsia, n(%)	47 (84.5)	48 (98)	0.03*	62.5%
Preeclampsia, n(%)	8 (14.5)	1 (2)		

* *Fisher test*

Table 3. hs-CRP distribution in case and control group

hs-CRP (mg/l)	Control (n:55) median (range)	Case (n:55) median (range)	p*
First Trimester	3.10 (02–102)	3.98 (0.32–21)	0.66
Second Trimester	3.97 (0.42–27.48)	4.07 (0.54–72.2)	0.88
Post Partum	5.52 (0.28–89.0)	7.47 (0.33–72.2)	0.52

**non-parametric (Mann-Whitney) test*

In the calculation of the General Linear Model (GLM) of repeated measurement was found significant differences in the increment of PAI-1 mRNA between the control and the case group ($p=0.02$) as in the chart above.

If both groups are combined and are performed comparative evaluation of subjects who became preeclamsia (Table 5), then will be found that hs-CRP levels are higher in preeclampsia, both the first trimester, second trimester and postpartum, but the statistics will not be significant.

Table 5. The distribution of hs-CRP in normal pregnancy and preeclampsia subjects

hs-CRP (mg/l)	Normal pregnancy (n:95) median (range)	Preeclampsia (n:9) median (range)	p
First Trimester	3.1 (0.2–30.33)	6.34 (1.27–102)	0,056
Second Trimester	3.83 (0.42–72.2)	7.87 (1.31–14.7)	0,053
Post Partum	5.85 (0.28–78.4)	13.4 (1.26–89)	0,91

Mann-Whitney test

DISCUSSION

In this study, it was found that the incidence of preeclampsia in population study was 8.6%, not much different from the incidence in the general population.[1,3]

Sociodemographic data in both groups of research subjects only different on variable of Body Mass Index (BMI) in first trimester, while the variables of age, history of hypertension, and smoking habits did not have differences.

This is important to analyze, considering preeclampsia was already proven to have a strong risk factor, especially a history of hypertension or previous preeclampsia and obesity.[1] In the case of it is obesity, often associated with increased systemic inflammation and insulin resistance that would increase preeclampsia.[1,18]

The habit of smoking had an inverse relationship with preeclampsia because it demonstrated a lower incidence of preeclampsia in pregnant women smokers. This is presumable related to the role of nicotine as a trigger to the release of nitric oxide by a receptor in the placenta and as reducer of free radical production.[19] The initial data before the treatment was proven to be equivalent in the control and case group.

Previous history of hypertension or preeclampsia should also be made equivalent to reduce confounding factors.[1]

The interesting thing in this study was Body Mass Index (BMI) variable was higher at baseline in the treated group than in, the control group, but had lower rates of preeclampsia. This gave an idea/opportunity that the administration of pro-antioxidant-enriched milk may reduce the incidence of preeclampsia, although in cases of excess weight. Of course this should be proved by a sharper research.[1,18,20-21]

Table 4. Distribution of PAI-1 in the control and case group

PAI-1 (genom-Equivalent)	Control (n:55) median (range)	Case (n:55) median (range)	p
First Trimester	251.63 (206.52–397.49)	238.56 (203.82–320.15)	0,016*
Second Trimester	506.64 (413.04–794.98)	477.19 (407.64–640.29)	0,007*
Post Partum	2026.57 (1264.63–3179.91)	1908.79 (1630.57–2561.18)	0,005*
$\Delta 2^{nd}$ Trimester - 1^{st} Trimester	255.78 (21.69–518.67)	229.85 (153.38–412.99)	0,09
ΔPost Partum- 1^{st} Trimester	1777.98 (1056.56–2934.15)	1660.24 (1387.05–2319.81)	0,01*
ΔPost Partum- 2^{nd} Trimester	1548.47 (713.35–2686.38)	1404.82 (1059.80–2094.39)	0,04*

**Nonparametric test (Mann-Whitney)*

Outcomes of neonatal birth weight and gestational age did not differ between control and case groups. After being evaluated, the reason was because of its relatively small numbers of subjects, especially the numbers of preeclampsia in the treated group, only 1 subject. With the pathogenesis of preeclampsia which primarily involves the process of placentation, then the possibility of stunted fetal growth and preterm births will increase.[1,5]

The pro-antioxidant-enriched formula feeding in this study gave evidence that it could reduce the incidence of preeclampsia from 14.5% to 2%. This is relevant with Rumiris et al study.[15]

Parameters in clinical trial that can also be assessed is the experimental event rate (EER) or the proportion of milk failure in preventing preeclampsia in case group which was 2%. While the control event rate (CER) or the failure of milk in preventing preeclampsia in control group was 14.5%.

Relative risk reduction (RRR) in this study was about 62.5%, meaning that the provision of infant formula enriched with pro-antioxidants decreased the rate of preeclampsia about 62.5% in pregnant women with low antioxidant.

Absolute risk reduction (ARR) or the incidence of factual preeclampsia between the control and case group was 12.5%.

Number needed to treat (NNT) or number of subjects that should be given milk to prevent one incidence of preeclampsia in this study were 8 pregnant women with low levels of antioxidants.

The mechanisms that underline the work of antioxidant administration in reducing the incidence of preeclampsia is not fully understood. One of which presumably is the improvement of systemic oxidative stress that occurs. These conditions of oxidative stress triggers inflammation and endothelial dysfunction, including platelet aggregation.[1]

In this study, the obtained hs-CRP plasma levels of both groups were not significant. This does not support the theory that linked the involvement of the inflammatory process that occurs with oxidative stress in preeclampsia.[5,7,10]

However, if the calculated statistics in both groups were combined and the variable hs-CRP was compared, it is clear that hs-CRP in subjects who suffer from preeclampsia is higher than in normal ended pregnancy, although the statistics was not significant. This is because the number of samples is less to compare the preeclampsia group (only 9 subjects from 104 subjects) with normal pregnancies.

Several clinical explanation on why the two groups, either treated or control were not different in levels of CRP is because the mechanism of hs-CRP production is heavily influenced by other inflammatory factors. On the subjects were made an exclusion of other infection such as respiratory tract infections, urinary tract infections and so on throughout the pregnancy.[22]

The interesting thing from this study was the tendency of hs-CRP to increase in the second trimester and postpartum compared to the first trimester. This explanation was proposed by Sibai et al, that pregnancy itself is an inflammatory condition, especially in third trimester.[1] So it can be said that this study also supports this theory. Preeclampsia happens in a severe/extreme systemic inflammatory conditions such that systemic endothelial damage will occur.[1]

The maternal systemic inflammation relation to the pathogenesis of preeclampsia has been widely studied several decades.[11] The explanation that can be adduced is debris of placental trophoblast escape into the maternal circulation.[23] Trophoblast debris is normally found in small amounts in the blood of pregnant women. In conditions of impared trophoblast invasion and increased oxidative stress, especially in preeclampsia, trophoblast apoptosis occurs much so that the amount of the released trophoblast into the maternal circulation is increased.[24] This trophoblast is semiallogenic (partly derived from the genetic paternal), would trigger maternal systemic inflammatory reactions.[25] Inflammatory mediators that are released will further increase trophoblast apoptosis so it will multiply trophoblast escape into the circulation, like a vicious circle.[26] Moreover, the process of oxidative stress in preeclampsia that occurs also triggers a systemic inflammatory process.[27] However, this study did not support the above theory.

This study found that the factor proven to be associated with the incidence of preeclampsia is cell-free mRNA PAI-1. This is similar to the results of Purwosunu et al study.[2] The control group, had significantly greater mRNA expression of PAI-1 genomic than the case group since the first trimester of pregnancy. The difference is consistent with the pathogenesis previously thought, namely the role of PAI-1 as a primary inhibitor of plasmin enzymes work in protein degradation in the process of remodelling the decidual spiralis arteries.[2]

This study also supports the theory which state that preeclampsia process has been started since the beginning of pregnancy (first trimester). Farina et al also showed that preeclampsia can be predicted at the age of 10-14 weeks, by examining specific angiogenesis factor mRNAs.[28]

The production of PAI-1 mPRNA was also found growing in the second trimester and postpartum when compared with the first trimester, either in treatment or control group. Explanation that could be found is physiological hypercoagulable state in pregnancy. The production of plasmin itself was also increased in hypercoagulable state of pregnancy. In the control group with higher PAI-1 in the second trimester and postpartum, preeclampsia were found many instances. The relationship of this event with preeclampsia is a possibility of spiral arteries and thrombosis leading to infarction of the placenta that have been proved partly occur in preeclampsia.[29] Spiral arteries thrombosis was also proven to be closely associated with endothelial dysfunction caused by increased oxidative stress in preeclampsia.[30] Endothelial dysfunction that occured resulting in increased endothelial cells permeability and platelet aggregation. Incidence of platelet aggregation is considered hypercoagulable and causing thrombosis.[30]

Another investigation in this study was the role of infant formula enriched with pro-antioxidants on the production of PAI-1. In this study, pro-antioxidant fortified milk to suppress the production of cell-free mRNA PAI-1 which is a genomic signal for the pro-

duction of PAI-1. This was proven by the difference in PAI-1 in second trimester-first trimester, postpartum-first trimester, as well as postpartum-second trimester between groups (Table 4), although there was significant statistical difference in postpartum-first trimester and postpartum-second trimester ($p<0.05$).

The mechanism of suppresion of mRNA production of PAI-1 done by the feeding of formula enriched with pro-antioxidant is assumed to suppress oxidative stress conditions[27] as a part of the pathogenesis of preeclampsia. The production of PAI-1 alone increased in systemic oxidative stress studied by Oszajca et al[4] and Furumaya et al.[31]

In this study, the differences in PAI-1 mRNA were also obtained to be different in the second trimester and postpartum between the control and case group. These results support several previous studies, especially Purwosunu et al. at the third trimester.[2] Mechanisms that could explain the release of placental mRNA into the maternal circulation in preeclampsia due to infarction of the placenta.[2] Trophoblast itself is still there in the placental bed spiral arteries until the puerperium completed, and suffered massive apoptosis. Purwosunu et al in another study was also to prove the same thing as postpartum, although only a portion of the mRNA of angiogenesis factors, not the PAI-1.[32]

From this study it can be concluded that feeding of formula milk enriched with pro-antioxidants can significantly decrease the incidence of preeclampsia in pregnant women with conditions of lower systemic antioxidant (FRAP < 900 μmol/l).

One of the pathophysiology as proven by this study is the role of PAI-1 produced by endothelial started in the first trimester where the implantation of trophoblast process and spiral artery remodeling begins. The feeding of formula milk enriched with pro-antioxidants can suppress the production of PAI-1 genomic mRNA so that the function of plasmin enzyme in the process of remodeling the spiral arteries will get better and in the second trimester the production of PAI-1 mRNA is suppressed, so that the incidence of platelet aggregation is decreased.

The role of inflammation as the pathogenesis of preeclampsia, characterized by hs-CRP, in this study there was an increasing trend, but it needs more subjects.

The advantages of this research are the increasing of complex pathogenesis of preeclampsia through the inflammation and the involvement of PAI-1 and the relating to oxidative stress as well as direct clinical interventions such as pro-antioxidant fortified milk that can be applied in the management of patients for prevention.

Limitations of this study was not examined other factors involved in the pathogenesis of preeclampsia as a complex of other oxidative stress enzymes such as Superoxide Dimutase (SOD), glutathione peroxidase (GPX), as well as the factors pro and anti-angiogenesis. The involvement of PAI-1 is only one part of the pathogenesis of preeclampsia. It required a more complex study, but certainly with larger subjects of research and fund.

REFERENCES

1. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005; 365: 785-99
2. Purwosunu Y, Sekizawa A, Koide K, Farina A, Wibowo N, Wiknjosastro GH. Cell-Free mRNA concentrations of plasminogen activator inhibitor-1 and tissue-type plasminogen activator are increased in the plasma of pregnant women with preeclampsia. Clinical Chemistry. 2007; 53: 399-404
3. Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia. Am J Obstet Gynecol. 1998; 179: 1359-75
4. Oszajca K, Bieniaz M, Brown G, Swiatkowska M, Bartkowiak J, Szemraj J. Effect of oxidative stress on the expression of t-PA, u-PA, i-PAR, and PAI-1 in endothelial cells. Biochem Cell Biol. 2008; 86: 477-86
5. Boutsikou T, Mastorakos G, Kyriakakou M, Margeli A, Hassiakos D, Papassotiriou I. Circulating levels of inflammatory markers in intrauterine growth restriction. Mediators of inflammation. 2010; 2010: 1-7
6. Wilkstrom A-K, Nash P, Eriksson UJ, Olovsson MH. Evidence of increased oxidative stress and a change in the plasminogen activator inhibitor (PAI)-1 to PAI-2 ratio in early-onset but not late-onset preeclampsia. Am J Obstet Gynecol. 2009; 201: 597.e1-8
7. Darcy B C, McDonald GB, Brateng D, Desai M, Thach CT, Easterling TR. The relationship between hemodynamics and inflammatory activation in women at risk for preeclampsia. Obstet Gynecol. 2001; 98: 1109-16
8. Boggess KA, Lieff S, Murtha AP, Moss K, Beck J, Offenbacher S. Maternal periodontal disease is associated with an increased risk for preeclampsia. Obstet Gynecol. 2003; 101: 227-31
9. Kumru S, Godekmerdan A, Kutlu S, Ozcan Z. Correlation of maternal serum high-sensitive C-reactive protein levels with biochemical and clinical parameters in preeclampsia Eur J Obstet Gynecol Reprod Biol. 2006; 124: 164-7
10. Ruma M, Boggess K, Moss K, Jared H, Murtha A, Beck J. Maternal periodontal disease, systemic inflammation, and risk for preeclampsia. Am J Obstet Gynecol. 2008; 198: 389.e1-e5
11. Qiu C, Luthy DA, Zhang C, Walsh SW, Leisenring WM, Williams MA. A prospective study of maternal serum C-reactive protein concentrations and risk of preeclampsia. Am J Hypertens. 2004; 17: 154-60
12. Djurovic S, Clausen T, Wergeland R, Brosstad F, Berg K, Henriksen T. Absence of enhanced systemic inflammatory response at 18 weeks of gestation in women with subsequent preeclampsia. Br J Obstet Gynaecol. 2002; 109: 759-64
13. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 (PAI-1) expression and activity in human aortic endothelial cells. Circulation. 2003; 107: 398-404
14. Orbe J, Rodriguez JA, Calvo A, Grau A, Belzunce MS, Martinez-Carob D. Vitamins C and E attenuate plasminogen activator inhibitor-1 (PAI-1) expression in a hypercholesterolemic porcine model of angioplasty. Cardiovascular Research. 2001; 49: 484-92
15. Rumiris D, Purwosunu Y, Wibowo N, Farina A, Sekizawa A. Lower rate of preeclampsia after antioxidant supplementation in pregnant women with low antioxidant status. Hypertens Pregnancy. 2006; 25: 241-53
16. Purwosunu Y, Sekizawa A, Okazaki S, Farina A, Wibowo N, Nakamura M. Prediction of preeclampsia by analysis of cell-free messenger RNA in maternal plasma. Am J Obstet Gynecol. 2009; 200: 386.e1-.e7
17. Harun SR, Putra ST, Chair I, Satroasmoro S. Uji Klinis. In: Sastroasmoro S, Ismael S, editors. Dasar-dasar metodologi penelitian klinis. Jakarta: Sagung Seto; 2010: p. 166-90
18. Wolf M, Kettyle E, Sandler L, Ecker JL, Roberts J, Thadhani R. Obesity and preeclampsia: the potential role of inflammation. Obstet Gynecol. 2001; 98: 757-62

19. Conde-Agudelo A, Althabe F, Belizan JM, Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: A systematic review. Am J Obstet Gynecol. 1999; 181: 1026-35
20. Mostello D, Chang JJ, Luehr JAL, Shyken J, Leet T. Recurrent preeclampsia: the effect of weight change between pregnancies. Obstet Gynecol. 2010; 116: 667-72
21. Getahun D, Ananth CV, Oyelese Y, Chavez MR, Kirby RS, Smulian JC. Primary preeclampsia in the second pregnancy: effect of changes in prepregnancy body mass index between pregnancy. Obstet Gynecol. 2007; 110: 1319-25
22. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix AJ. Serum Procalcitonin and C-Reactive Protein Levels as Markers of Bacterial Infection: A Systematic Review and Meta-analysis. Clinical Infectious Diseases. 2004; 39: 206-1
23. Cotter AM, Martin CM, O'Leary JJ, Daly SF. Increased fetal DNA in the maternal circulation in early pregnancy is associated risk of preeclampsia. Am J Obstet Gynecol. 2004; 191: 515-20
24. Ishihara N, Matsuo H, Murakoshi H, Laoag-Fernandez JB, Samoto T, Maruo T. Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. Am J Obstet Gynecol. 2001; 186: 158-66
25. Levine RJ, Qian C, Leshane ES. Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia. Am J Obstet Gynecol. 2004; 190: 707-13
26. Kwaan HC, Wang J, Boggio L, Weiss I, Grobman WA. The thrombogenic effect of an inflammatory cytokine on trophoblasts from women with preeclampsia. Am J Obstet Gynecol. 2004; 191: 2142-7
27. Moretti M, Phillips M, Abouzeid A, Cataneo RN, Greenber J. Increased breath markers of oxidative stress in normal pregnancy and in preeclampsia. Am J Obstet Gynecol. 2004; 190: 1184-90
28. Farina A, Zucchini C, Sekizawa A, Purwosunu Y, Santis Pd, Santarsiero G. Performance of messenger RNAs circulating in maternal blood in the prediction of preeclampsia at 10-14 weeks. Am J Obstet Gynecol. 2010; 203: 575.e1-7
29. Redman CW. Platelets and the beginning of preeclampsia. N Eng J Med. 1990; 323: 478-80
30. Wang Y, Gu Y, Zhang Y, Lewis DF. Evidence of endothelial dysfunction in preeclampsia: decreased endothelial nitric oxide synthase expression is associated with increased cell permeability in endothelial cells from preeclampsia. Am J Obstet Gynecol. 2004; 190: 817-24
31. Furuyama A, Hirano S, Koike E, Kobayashi T. Induction of oxidative stress and inhibition of plasminogen activator inhibitor-1 production in endothelial cells following exposure to organic extracts of diesel exhaust particles and urban fine particles. Arch Toxicol 2006; 80: 154-62
32. Purwosunu Y, Sekizawa A, Farina A, Wibowo N, Koide K, Okazaki S. Evaluation of physiological alterations of placenta through analysis of cell-free messenger ribonucleic acid concentrations of angiogenic factors. Am J Obstet Gynecol. 2008; 198: 124.e1