File Comparison Report

Produced by WCopyfind.4.1.4 with These Settings:

Shortest Phrase to Match: 3
Fewest Matches to Report: 2
Ignore Punctuation: No
Ignore Outer Punctuation: No
Ignore Numbers: No
Ignore Letter Case: No
Skip Non-Words: No
Skip Long Words: No
Most Imperfections to Allow: 4
Minimum % of Matching Words: 85

Perfect Match	Overall Match	View Both Files	File L	File R
69 (3% L, 3% R)	76 (3%) L; 73 (3%) R	Side-by-Side	02.Differences_in_Mean.docx	01.Analyses_of_Nutrients.docx
107 (6% L, 5% R)	109 (6%) L; 112 (5%) R	Side-by-Side	03.Differences_in_Malondial.docx	01.Analyses_of_Nutrients.docx
89 (5% L, 4% R)	94 (5%) L; 95 (4%) R	Side-by-Side	03.Differences_in_Malondial.docx	02.Differences_in_Mean.docx
64 (4% L, 3% R)	68 (4%) L; 64 (3%) R	Side-by-Side	04.Differences_in_brain.docx	01.Analyses_of_Nutrients.docx
48 (3% L, 2% R)	51 (3%) L; 53 (2%) R	Side-by-Side	04.Differences_in_brain.docx	02.Differences_in_Mean.docx
77 (5% L, 4% R)	84 (5%) L; 78 (4%) R	Side-by-Side	04.Differences_in_brain.docx	03.Differences_in_Malondial.docx

WCopyfind.4.1.4 found 6 matching pairs of documents.

Research Article

Differences in Mean Levels of Maternal Resistin Serum between Early Onset Preeclampsia (EOPE) and Late Onset Preeclampsia (LOPE)

Research Article
Differences in Mean Levels of Maternal Resistin Serum between Early Onset Preeclampsia (EOPE) and Late Onset Preeclampsia (LOPE)

INTRODUCTION

Preeclampsia is one of the *major* causes of morbidity and mortality of the mother and fetus. World Health Organization (WHO) reported the number of deaths caused by preeclampsia by 16% in developing countries . Preeclampsia resulting 3-25 fold increased risk obstetric complications and is the cause of 30-40% of perinatal deaths in Indonesia [1] . The incidence of preeclampsia ranges between 5-10% of all pregnancies . Incidence of preeclampsia in the United States, Canada and Western Europe ranges between 2-5% of all pregnancies and higher, 4-18% in some developing countries in Africa [1] . The incidence of preeclampsia in Indonesia ranged between 3-10% [3] . In Dr.M. Djamil General Hospital in Padang during the [2] year 2011, the incidence of preeclampsia was 8.31%, on 2012 was 11.47% and on 2013 was 12.02%.

Preeclampsia is divided into early onset preeclampsia (EOPE) (<34 weeks) and late onset preeclampsia (LOPE) (>34 weeks) of pregnancy based on the onset of clinical manifestations of preeclampsia. The EOPE and LOPE has a different pathogenesis. The PEAD is often associated with impaired uteroplacental perfusion caused by disruption of trophoblast invasion, while LOPE is *often* associated with the presence of extrinsic and maternal factors .

Preeclampsia is a protean syndrome, in which multiple [4,5] organ systems can be affected compared to the others. Preeclampsia is *mainly* characterized by hypertension and proteinuria or may be associated with abnormalities in laboratory test results that renal function, hepatic or hemostasis after 20 weeks of pregnancy .

Analysis of risk factor for preeclampsia is needed to [1,6] mitigate the adverse effects of preeclampsia. One among of these risk factor is resistin which is associated with inflammation and insulin resistance, so that resistin was a

maternal risk factor that associated with LOPE

.

Resistin is an adipose tissue-specific secretory factor [7] (ADSF), a hormone secreted by adipose tissue that induces insulin resistance in muscle and liver. Resistin stimulated by inflammatory conditions that produce proinflammatory cytokines

.

Preeclampsia is associated with inflammation and insulin resistance [7,8] which is affected by resistin. Resistin is associated with late onset preeclampsia because it was a maternal factors, so there was an increasing of maternal resistin serum levels in late onset preeclampsia

.

Previous study reported an elevated of maternal resistin serum levels associated with a systemic inflammatory response and insulin resistance which is both of them are [2] [2] increased in preeclampsia compared to normal pregnancy

.

The other study showed the mean of Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and high sensitivity C-reactive protein (hs-CRP) was higher in late onset preeclampsia than early onset preeclampsia group. From various studies on the above it could be estimated that the inflammatory factor and insulin resistance are associated with higher levels of resistin more dominant in late onset preeclampsia group compared to early onset preeclampsia group.

The HOMA-IR could be used as an indicator of insulin resistance, but not for inflammatory states, contrary hs-CRP could be used as an indicator of inflammation, but not for insulin resistance. Resistin is expected to describe both of them, because it was associated with insulin resistance and inflammation [7]

.

MATERIALS AND METHODS

This study is an analytical cross sectional study with 20 women of early onset preeclampsia (EOPE) and 20 women of late onset preeclampsia (LOPE) who met the inclusion criteria and there were no exclusion criteria. The samples were recruited in Dr.M. Djamil General Hospital, Padang from July-October, 2015.

The inclusions criteria were women who detected early onset preeclampsia or late onset preeclampsia in obstetrics emergency ward of Dr.M. Djamil General Hospital, Padang and willing to follow the research for taking blood samples. The exclusions criteria were diabetes mellitus (random blood

sugar as >200 mg dLG
), had suffered coronary heart disease,
chronic kidney disease, chronic liver disease and obesity
(BMI before pregnancy >25 kg mG
1
).
Maternal resistin serum was examined by enzyme-linked
2
immunosorbent assay (ELISA) method in Biomedic Laboratory
in Medical Faculty of Andalas University. Statistical analysis
was conducted by using SPSS program 20th version. The data
distribution was normal with p>0.05. The differences mean
levels of maternal resistin serum was analyzed by using
independent t-test.

RESULTS

Characteristics of research subjects: Forty patients of
research subjects consisted 20 patients of early onset
preeclampsia (EOPE) and 20 patients of late onset
preeclampsia (LOPE). Mean levels of maternal age in EOPE
group is 34.4±5.144 years, whereas in LOPE group is
32.9±6.324 years with p = 0.416. According to maternal age
9-12

Table 1: Characteristics of research subjects between early onset preeclampsia and late onset preeclampsia

Characteristics	EOPE (n = 20)	LOPE (n = 20)	Total (%)	p
Maternal age group				
<20 years	0 (0%)	0 (0%)	0	
20-35 years	8 (40%)	10 (50%)	45	0.537
>35 years	12 (60%)	10 (50%)	55	
Maternal age (0±SD) years	34.4±5.144	31.9±6.314		0.416
Gravidity group				
Primigravid	6 (30%)	7 (35%)	32.5	0.744
Miltigravid	14 (70%)	13 (65%)	67.5	
Gravidity (0±SD)	2.4±1.314	2.65±1.461		0.573
BMI group				
Underweight	0 (0%)	0 (0%)	0	
Normoweight	14 (70%)	12 (60%)	65	0.520
Overweight	6 (30%)	8 (40%)	35	
BMI (0±SD)	22.55±1.595	22.59±1.812		0.946

Table 2: Mean levels of maternal resistin serum in early onset preeclampsia and
late onset preeclampsia

EOPE		LOPE	
Variable			
Mean	SD	Mean	SD
Maternal resistin serum (ng mLG 1)	2.526	1.603	8.891	6.219

Table 3: Mean levels difference of maternal resistin serum in early onset
preeclampsia and late onset preeclampsia

Variable	Mean difference	CI (95%)	p
Maternal resistin serum (ng mLG 1)	6.365	3.3835-9.3475	0.000

groups, study of subjects in early onset preeclampsia
consists 8 patients (40%) in group of maternal age

20-35 years old, 12 patients (60%) in group of maternal age >35 years old, none in group of maternal age <20 years old. Research subjects of late onset preeclampsia consists of 1 0 patients (50%) in group of maternal age 20-35 years old, 10 patients (50%) in group of maternal age >35 years old, none in group of maternal age <20 years old.

Mean levels of gravidity in early onset preeclampsia (EOPE) group is 2.4±1.314, whereas in late onset preeclampsia (LOPE) group is 2.65±1.461 with p = 0.573. According to gravidity groups, study of subjects in early onset preeclampsia consists 6 patients (30%) in group of primigravida, 14 patients (70%) in group of multigravida. Research subjects of l ate onset preecl ampsi a c onsi sts of 7 patients (35%) in group of primigravida, 13 patients (65%).

Mean levels of Body Mass Index (BMI) in early onset preeclampsia (EOPE) group is 22.551±1.595, whereas in late onset preeclampsia (LOPE) group is 22.588±1.4812 kg mG with p = 0.946. According to BMI groups, study of subjects in EOPE consists 14 patients (70%) in group of normal weight, 6 patients (30%) in group of overweight, none in group BMI underweight. Research subjects of late onset preeclampsia consists 12 patients (60%) in group of normal weight, 8 patients (40%) in group of overweight, none in group BMI underweight.

2

3

There were no statistically significant differences regarding maternal age, group of maternal age, gravidity, group of gravidity, BMI and group of BMI characteristic between those two groups with p>0.05 showed in Table 1.

Mean levels of maternal resistin serum in early onset preeclampsia and late onset preeclampsia: Mean levels of maternal resistin serum was higher in late onset preeclampsia vs

2.526±1.603 ng dLG

than early onset preeclampsia (8.891±6.219 ng mLG

1

), showed in Table 2.

Differences in mean levels of maternal resistin serum between late onset preeclampsia and early onset preeclampsia: The data distribution was normal with Kolmogorov Smirnov test. Analysis was performed with independent t-test. Table 3 showed the differences in mean levels of maternal resistin serum between early onset preeclampsia and late onset preeclampsia as 6.365 ng mLG with Confidence Interval (CI) 95% 3.384-9.347. There is a high significant differences with p<0.001.

DISCUSSION

The mean levels of *maternal* serum resistin in early onset preeclampsia (EOPE) group was 2.526±1.603 ng mLG whereas in late onset preeclampsia (LOPE) group was 8.891±6.219 ng mLG

(Table 2), with a mean difference

6.365 ng dLG

1
1
, 95% CI 3.384-9.347. Statistical test showed p<0.001, that the mean levels of *maternal* serum resistin significantly was higher in LOPE than EOPE (Table 3). Resistin is a hormone secreted from adipose tissue that induces insulin resistance in muscle and liver. Resistin stimulated by inflammatory conditions that produce proinflammatory cytokines. Resistin contribute to the inflammatory disorders such as coronary heart disease,
1
1
1
chronic kidney disease and liver cirrhosis as well as insulin resistance in diabetes mellitus through the activation of proinflammatory cytokines varied which depending on the organs affected
.
Insulin resistance and inflammation were the condition
7,8
associated to preeclampsia. This factor related to the role of resistin through the release of proinflammatory cytokines. The relationship between resistin and preeclampsia is connected by those which is a maternal factor in preeclampsia. Therefore, resistin is associated to late onset preeclampsia, so the levels of maternal resistin serum was increased in late onset preeclampsia
.
Inflammation and insulin resistance have been
7,8
investigated, that the comparasion between HOMA-IR and hs-CRP in EOPE and LOPE. This study reported the results that mean levels of HOMA-IR and hs-CRP were higher in LOPE compared to EOPE (HOMA IR: 4.86±5.50 vs 3.99±5.97 and hsCRP: 123.08±38.67 vs 26.54±34.7 mg LG
). This study suggested that the inflammatory factor and insulin resistance were more dominant in LOPE compared to EOPE.
1
A s i mi l ar r es ul t s w as r epor t ed i n whi c h t he mean l evel s of maternal resistin serum was higher in preeclampsia compared to normal pregnancy related to an increase in insulin resistance and the response systemic inflammation that happened in preeclampsia particularly late onset preeclampsia
.
A cross sectional study in which two groups of pregnant
9-12
women with preeclampsia (n = 15) and normal pregnancy (n = 23) demonstrated th mean levels of maternal resistin serum was higher in preeclampsia (5.68±0.41 ng mLG
)
compared to normal pregnancy (4. 65±0. 32 ng mLG
),

p = 0.028. The mean levels of maternal resistin serum related to the mean of HOMA-IR and proinflammatory cytokines that were elevated in preeclampsia compared to normal pregnancy. T he mean of H OMA-I R (2. 5±0. 8 vs 1. 4±0. 1), IL-6 (6.34±1.02 vs 2.80±0.31) and TNF-" (1.89±0.18 vs 1.23±0.10)
.
Another cross-sectional study on two groups of
9
pregnant women, which were preeclampsia (n = 29) and normal pregnancy (n = 30), found that mean levels of maternal resistin serum was higher in preeclampsia 61.98±32.26 ng dLG
, compared to normal pregnancy 38.06±31.26 ng dLG
1
1
, p = 0.013. Insulin resistance is thought related the increasing the levels of maternal resistin serum, because the mean of HOMA-IR was higher in preeclampsia compared to normal pregnancy (4:44±4:02 vs 3.99±2.82)
.
A cross sectional study was conducted on two groups of pregnant women, which are preeclampsia (n = 50) and normal pregnancy (n = 50). The median levels of maternal resistin
1
1
10
4
serum were higher in preeclampsia 61 ng mLG
compared to
normal pregnancy 25.5 ng mLG
1
1
, p = 0.033. Insulin resistance is thought related the increasing the levels of maternal resistin serum, because the median of HOMA-IR was higher in preeclampsia compared to normal pregnancy (4.7 vs 3.6)
.
A cross sectional study was conducted on two groups of pregnant women, which are preeclampsia (n = 16) and normal pregnancy (n = 22). The mean levels of maternal resistin serum was higher in preeclampsia (12.06±0.973 ng mLG
)
compared t o n ormal p regnancy (7. 35±1. 195 ng mLG
),
p = 0.041. Inflammatory factors is thought related the increasing the levels of maternal resistin serum in preeclampsia. There was an increasing mean levels of TNF" in preeclampsia compared to normal pregnancy (15.23±0.674 vs 12.84±0.348 ng mLG
1
, p = 0.021)
. Based on

that description, there was a correlation between previous studies and this study
9-12
12
.
The mean levels of HOMA-IR and hs-CRP were higher in late onset preeclampsia than early onset preeclampsia, so according to those studies above, the insulin resistance and inflammation that were higher in late onset preeclampsia than early onset preeclampsia related to the levels of maternal resistin serum that was higher in late onset preeclampsia than early onset preeclampsia. It was appropriate with the results of this research that the mean levels of maternal resistin serum in late onset preeclampsia was significantly higher than early onset preeclampsia with $p<0.001$.
CONCLUSION
The mean levels of maternal resistin serum was higher significantly in late onset preeclampsia (LOPE) compared to early onset preeclampsia (EOPE).
11
1
1

Analyses of Nutrients and Body Mass Index as Risk Factor for Preeclampsia

Introduction

Preeclampsia is an important problem in obstetrics because it is still a major cause of maternal mortality compared to bleeding and infection. Preeclampsia leads to maternal and perinatal morbidity. Preeclampsia is *also* associated with high rates of preterm delivery, small for gestational ages, and perinatal death [1]. Little is known about the patho- genesis of preeclampsia. Many factors are identified as risk factors for preeclampsia including parity, multiple preg- nancies, age, family history of preeclampsia, obesity, his- tory of systemic disease, and nutrition.

Since preeclampsia is characterized by reduced perfu- sion of the placenta, oxidative stress, and endothelial dys- function, nutrition has long been hypothesized to have a role in the etiology of preeclampsia [2]. Oxidative stresses are proposed as the linkage between the two stages of preeclampsia. Nutrients can affect oxidative stress by increasing or decreasing free radicals or antioxidants or by providing substrate for the formation of free radicals. Several nutrients, particularly omega-3 (n - 3) fatty acids, antioxidants, and folic acid, have an important roles in modulating endothelial function. It has also been suggested that nutrients such as trace elements, fatty acids, and folic acid can contribute to insulin resistance, a risk factor for preeclampsia. In many studies, decrease in serum magne- sium levels has been considered as the cause of patho- genesis of preeclampsia. Minerals have an important

influence on the health of pregnant women and growing fetus. Among them, serum or placental zinc (Zn) concen- trations have been reported to be low in PE women. Fur- thermore, decreased levels of zinc, selenium, and copper have been observed in patients with preeclampsia [3]. In another side, the nutrients with antioxidants among high risk women showed a protective effect [4]. Folic acid has been hypothesized as a protective agent of preeclampsia.

Maternal obesity and insulin resistance are also believed to be important risk factors for the development of pla- cental endothelial dysfunction and preeclampsia. Preven- tion of preeclampsia has remained elusive, owing largely to their complex nature. Currently, maternal obesity in prepregnancy is one of the strongest modifiable risk fac- tors. Recent studies have shown a relation between obesity in prepregnancy and the risk of preeclampsia. The reason for obesity being associated with an increased risk of preeclampsia was explained by increased levels of serum triglycerides and very low-density lipoprotein particles in obese women. This lipid alterations have been suggested to promote oxidative stress caused by ischemia–reperfusion mechanism or activated neutrophils, which leads to endothelial cell dysfunction [1].

The hypothesis about nutritional status and body mass index (BMI) prepregnancy associated with preeclampsia has intrigued us to study the risk factor for preeclampsia in Dr. M. Djamil Hospital, Padang, Indonesia.

Methods

This was a case–control study at the Department of Obstetric and Gynecology in Dr. M. Djamil Hospital, Padang, Indonesia, between January and December 2013. Pregnant women after 20 weeks gestations were included. A total of 140 patients were enrolled in this study with 70 cases and 70 controls. Cases were those diagnosed with preeclampsia; meanwhile, controls were normotensive pregnant women without any other comorbidity.

After providing written informed consent, all subjects completed an interview for their nutritional status and prepregnancy BMI after delivery. The nutritional status was assessed by Food Frequency Questionnaire (FFQ) and then analyzed by Nutrisurvey Program. Calories, protein, fat, carbohydrates, calcium, phosphorus, zinc, sodium, potassium, magnesium, vitamin A, folic acid, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, vitamin C, and vitamin E were assessed. Maternal BMI was catego- rized into two groups: normal BMI and abnormal BMI. Normal BMI was defined as 18.5–24.9 kg/m2. The inde- pendent samples t test was used for nutritional status, and

Chi-square test was used for BMI. Odds ratio (OR) with

95% CI was calculated. A p value \0.05 was considered

statistically significant. For the nutrition variable, if the p value \0.25 then continued by logistic regression back- ward to assess the risk factor.

Results

Most of the subjects had normal weight, both in case and control groups. Table 1 shows that prevalence of abnormal BMI was more common in the preeclampsia group com- pared with those without preeclampsia 19 (27.1%) versus

12 (17.1%). BMI was not found as a significant risk factor in this study (p = 0.222).

As shown in Table 2, the mean level for most of the
variables, except calories, fat, and vitamin B1, was lower in subjects with preeclampsia than those without preeclamp- sia. The difference for most of the subjects was also sta- tistically significant with p \ 0.05, except for vitamin B1 and vitamin B2. Table 3 shows the risk factors for preeclampsia obtained by logistic regression analyses. The nutrients that were significantly associated with increased risk of preeclampsia were deficiency of vitamin E, zinc, fat, calcium, and vitamin C. Excess of calories and carbohy- drate also significantly associated with increased risk of preeclampsia. Meanwhile, vitamin A and vitamin B1 were protective factors.

Discussion

The reason for obesity being associated with an increased risk of preeclampsia was explained by increased levels of serum triglycerides, very low-density lipoproteins, and formation of small low-density lipoprotein particles in obese women. This lipid profile was also found in women with preeclampsia. These lipid alterations have been suggested to promote oxidative stress, caused by ische- mia–reperfusion mechanism or activated neutrophils, which leads to endothelial cell dysfunction [1]. Moreover, dyslipidemia also can cause atherothrombosis and induce the aggregation of the thrombocytes than can lead to coagulopathy which is a characteristic of preeclampsia.

Obesity is accompanied by oxidative stress. The origin of oxidative stress is proposed to be secondary to increased free fatty acids and inflammation. It is also suggested that diet can contribute to oxidative stress. Obese individuals have lower blood concentrations of antioxidants. This could be due to reduced dietary intake of antioxidants, but increased consumption by reactive oxygen species is also possible [5].

In this study, we found no relationship between BMI with preeclampsia. As mentioned before, dyslipidemia is the important factor that can lead into preeclampsia. But our study did not assess profile lipid of the subjects. Fur- thermore, central obesity has a higher risk of preeclampsia. Central obesity is characterized by visceral fat. Visceral fat produces C-reactive protein (CRP), PAI-1, and leptin that contributes to oxidative stress. People with central obesity have a higher risk to get preeclampsia. In this study, we did not assess the central obesity of the subjects. Measures of body composition, including percent body fat, may very likely identify the obese woman at risk of preeclampsia more accurately.

Our study found that carbohydrate intake in preeclampsia *group* was significantly higher than the non-preeclampsia group. The subjects that have higher carbo- hydrate will have lower protein level. Meanwhile, protein is needed in the process of trophoblast invasion so the protein-energy malnutrition increases the risk of preeclampsia. We also found that intake of proteins in preeclampsia was significantly lower in the preeclampsia group.

Folic acid and vitamin B12 are also a protector factors against preeclampsia. Those micronutrients play an important role in suppressing the metabolism of homo- cysteine, whereas the excess of homocysteine was a cau- sative factor of endothelial damage and became one of the causes of preeclampsia. In addition, folic acid- supple- ments preconception enhance the placentation process and can prevent preeclampsia. Three earlier cohort studies assessed the effect of folic acid containing multivitamins (including folic acid) and gestational hypertension (in- cluding preeclampsia), and all showed a protective effect of folic acid supplementation on preeclampsia [2, 6, 7].

Table 1 Asssociation between body mass index and the risk of preeclampsia

BMI
Preeclampsia
%
Non-preeclampsia
%
p*
OR
CI 95%
Min.
Max.
Abnormal BMI
19
27.1
12
17.1

0.222
1.801
0.797
4.067
Normal BMI
51
72.9
58
82.9
70
100
70
100
BMI body mass index, OR odds ratio, CI confidence interval
* p value were obtained by Chi-square
Table 2 Differences of mean nutritions level in preeclampsia and non-preeclampsia group
Preeclampsia
Mean (95% CI)
Non-preeclampsia
Mean (95% CI)
p*
Calorie (Kcal)
1269.22 (745.2–2214)
1171.15 (649.1–2447.6)
0.048
Protein (g)
42.37 (23.03–95.42)
54.9 (24.2–200.6)
0.000
Fat (g)
26.8 (4.65–58.9)
32.4 (10.79–93.12)
0.009
KH (g)*
219.5 ± 56.8
163.49 ± 46.5
0.000
Calcium (mg)
234.7 (63.7–758.3)
9362.9 (63.7–922.4)
0.000
Phosphor (mg)
589.4 (296.5–1444.5)
714.6 (324.6–2353.4)
0.006
Fe (mg)
9.4 (2.84–23.9)
11.06 (2.84–35.6)
0.005
Zinc (mg)
4.8 (2.39–12.87)
5.5 (2.4–16.5)
0.005
Natrium (mg)

299.3 (76.9–970.24)
533.3 (105.9–1847.60)
0.000
Kalium (mg)*
595.4 ± 237.2
732.15 ± 367.8
0.010
Magnesium (mg)
181.1 (97.1–370.14)
210.0 (97.1–554.5)
0.010
Vitamin A (lg)
544.9 (174.9–1567.4)
763.9 (174.9–3354.5)
0.000
Folic acid (lg)
108.6 (56.8–421.6)
163.5 (56.83–497.9)
0.000
Vitamin B1 (mg)
0.7 (0.27–2.15)
0.65 (0.27–2.01)
0.085
Vitamin B2 (mg)
0.94 (0.25–2.45)
1.1 (0.25–2.7)
0.046
Niacin (mg)
4.3 (0.6–11.9)
5.8 (2.1–23.7)
0.000
Vitamin B6 (mg)
1.1 (0.66–2.14)
1.22 (0.67–3.8)
0.009
Vitamin B12 (mg)
1.1 (0.15–9.15)
2.8 (0.27–21.1)
0.000
Vitamin C (mg)
43.4 (8.4–136.8)
68.2 (19.2–262.7)
0.000
Vitamin E (mg TE)
1.9 (0.2–4.4)
3.6 (1.8–8.2)
0.000
CI confidence interval
* p value was obtained by independent samples t test

A recent large cohort study from Denmark also showed that regular use of folic acid in pregnancy *was* related to a reduced risk of preeclampsia among normal-weight women [8]. But in this study, we did not found the folic acid and vitamin B12 as a protective agent of preeclampsia. The different result maybe caused by supplementation that has been done generally. In other side, the no supplementation subject was rare so that selection bias/confounding become difficult to control.

The previous study showed that vitamin B6 deficiency is associated with cardiovascular disease. This is related to the function of B6 for the establishment of pyridoxal 5 phosphate which is an essential coenzyme in many meta- bolism processes. The coenzyme deficiency will lead to the diseases such as preeclampsia. Another micronutrients that play a role in the occurrence of preeclampsia is magne- sium. In a study, obtained serum *magnesium* levels in patients with preeclampsia were significantly lower than normal pregnancy. This indicates that magnesium as one of the predictors of preeclampsia. Magnesium also allegedly acted as a coenzyme intracellular and functions to maintain contraction and vascular tone [2].

Serum zinc, calcium, and magnesium levels have been compared between preeclamptic and healthy pregnant women in various studies. Some results showed that copper, zinc, and
calcium levels were significantly lower in preeclamptic patient, whereas magnesium concentrations showed no sig- nificant differences between the two groups [9]. Our study is consistent with that study, whereas we found zinc and calcium deficiency as a risk factor for preeclampsia (OR 99.46, 95% CI
1.37, 7219; OR 31, 95% CI 1.21, 841).

Our study found that vitamin E and A deficiency increase the risk of preeclampsia (OR 19.57, 95% CI
2.5, 151, OR 1768, 95% CI 10.22, 305,890). Antioxi-
dants are important in maintaining cellular integrity in a normal pregnancy by inhibiting peroxidation reaction and thus protecting enzymes, proteins, and cells from destruction by peroxides. Antioxidant defense mecha- nisms include cellular and extracellular enzymes such as free radical scavengers, including vitamin C and E, and metabolites such as bilirubin and uric acid. Vitamin C and E are antioxidants derived from the diet. Vitamin C scavenges free radicals in the aqueous phase, and the lipid-soluble vitamin E acts in vivo to prevent the for- mation of lipid peroxides and thus protect cell mem- branes. There is evidence of oxidative stress in women with established preeclampsia, including increased plasma concentration of 8-epi-prostaglandin F2a, lipid peroxides, and decreased concentrations of antioxidants such as vitamins C and E [10].

Table 3 Results of multivariate logistic regression analysis

	OR	p	95% confidence interval
Calorie	3.87	0.028	2.39 ± 6,268,293
Protein	0.049	0.650	0.27 ± 12,994
Fat	59.182	0.138	3.14 ± 500
KH	52.9	0.063	0.801 ± 3495
Calcium	109	0.119	0.29 ± 40,041
Fe	9.84	0.149	0.441 ± 219
Zinc	99.4	0.035	1.37 ± 7219
Natrium			

0.62
0.731
0.041 ± 9.37
Kalium
0.005
0.054
0.000 ± 1.08
Magnesium
0.015
0.528
0.000 ± 7321
Vitamin A
0.002
0.055
0.000 ± 1.14
Folic acid
42.9
0.193
0.15 ± 12,383
Vitamin B1
0.000
0.009
0.000 ± 0.014
Vitamin B2
3.75
0.550
0.049 ± 285
Niacin
2.39
0.068
0.571 ± 1E ? 007
Vitamin B6
0.299
0.591
0.004 ± 24.3
Vitamin B12
4.511
0.243
0.36 ± 56.4
Vitamin C
19.5
0.004
2.52 ± 151
Vitamin E
1.76
0.004
10.2 ± 30.5
OR odds ratio
Conclusions
Deficiency of vitamin E, zinc, fat, calcium, and vitamin C, and excess of calories and carbohydrate were associated with increased risk of preeclampsia.

Differences in Malondialdehyde and Catalase Activity Levels
Between Abortion and Normal Pregnancy

INTRODUCTION

Abortion is defined as a termination of a pregnancy before
20 weeks of gestation or when the fetal weight is less than
500 g. Clinically, the most frequent abortion encountered in the hospital is incomplete abortion. Patients usually come with bleeding and severe abdominal pain. The second one is threatened abortion. Threatened abortion is the most common complication in the first half of pregnancy and have incidence about 20-25%. Less than 30% of the women who experience threatened abortion will end in spontaneous abortion1.

There are many factors that associated with abortion, so it is hard to determine the exact mechanism. In spite of many possibilities, there is now a clear evidence that abortion is associated with placental oxidative stress. The abnormal placentation will lead to placental oxidative stress with resultant destructive effects on the syncytiotrophoblast. The theory has been proposed as a mechanism involved in the etiopathogenesis of abortion2.

The body has many antioxidant systems to defend the excessive Reactive Oxygen Species (ROS) production3. In a healthy body, ROS and antioxidant remain in balance. When the body has an excessive amount of ROS, Oxidative Stress (OS) then occurs. An increase in the expression of oxidative stress marker in the trophoblast was detected in abortion and this was speculated to be a cause of early pregnancy loss4.

Antioxidant system divided into enzymatic and nonenzymatic group. Catalase (CAT) is one of enzymatic antioxidant that can remove hydrogen peroxide (H2O2), prevents lipid peroxidation in the cell membrane and works as free radicals binding5. The CAT role in pregnancy is very important, in early pregnancy CAT plays a role for implantation by protecting the blastocysts from superoxide radicals in the endometrium. The CAT is also important for embryonic development and the maintenance of early pregnancy. The CAT level was found increased during pregnancy and low CAT activity in plasma or placenta found in cases of spontaneous abortion6. Another study also suggests that low antioxidant level increases the risk of spontaneous abortion7.

Free radicals are thus highly unstable molecules that have electrons available to react with a various organic substrate such as lipids, proteins and DNA8. Accumulation of lipid peroxides may cause not only tissue damage but also some biological events to accelerate the termination of pregnancy. It has been reported that ROS or lipid peroxide stimulate synthesis of PGF2α that causes uterine contraction6. Oxidative stress has been variably determined by many ways. The most reliable assessment of free radicals synthesis is using assays directly to detect the superoxide or other free radical. However, this technique is difficult and require specialized document. In another side, measurement of the products of oxidative modification product provides the most direct and possible assessment of oxidative stress. Malondialdehyde
(MDA) is a metabolite of lipid peroxides that detectable in plasma and was used as an indicator of lipid peroxidation9. This study was carried out to estimate the mean difference of CAT and MDA in normal pregnancy, threatened abortion and incomplete abortion.

MATERIALS AND METHODS

This was an observational study with cross-sectional design on February-December, 2014. The population was all pregnant women before 20 weeks gestations were enrolled in the maternity ward of Dr. M. Djamil Hospital and Dr. Reksodiwiryo Hospital, Padang, Indonesia. Subjects were taken by consecutive sampling method. Cases were those diagnosed with threatened and incomplete abortion by a specialist doctor. Meanwhile, controls were normal pregnant women without any other comorbid.

Exclusion criteria were a mother with hydatidiform mole, uterus disorder, myoma, history of provocating and recurrent abortion. After providing *informed*, written consent, subject's blood was taken from antecubital vein for analyzed the CAT activity and MDA concentration with spectrophotometry method in Biomedical Laboratory of Medical Faculty of Andalas University.

The significant difference among the three groups was analyzed with ANOVA test. If $p<0.05$ was found, then the analysis continued with post hoc multiple comparison test to determined the differences between two group. Statistical analysis was conducted using a computer program.

RESULTS

During the study period, 42 blood samples were collected, consisting of 14 subjects with threatened abortion, 14 subjects with incomplete abortion and 14 subjects with normal pregnancy before 20 weeks gestations. Table 1 shows the activity of CAT enzyme in incomplete abortion group was
51.10±12.20 IU mgG1, the threatened abortion group was

66.46±9.11 IU mgG1, whereas normal pregnancy group was
78.26±8.88 IU mgG1. The analysis with statistical tests ANOVA found that p<0.001. To saw the specific difference between two subjects, the analysis continued by post hoc multiple comparisons test. As presented in Table 2, there were significant differences of CAT activity between incomplete abortion group and threatened abortion (p<0.001), incomplete abortion and normal pregnancy (p<0.001), threatened abortion and normal pregnancy (p = 0.01).

Table 1: Difference of catalase activity in incomplete abortion, threatened
abortion and normal pregnancy Catalase activity

Parameters	Mean±SD (IU mgG1)	95% CI	p-value*
Incomplete abortion	51.10±12.20	44.06-58.15	<0.001
Threatened abortion	66.46±9.11	61.20-71.72	
Normal pregnancy	78.26±8.88	73.13-83.38	

*p-values are obtained by ANOVA test, SD: Standard deviation, CI: Confidence interval

Table 2: Post hoc test for the difference of the mean level of catalase activity
in incomplete abortion, threatened abortion and normal pregnancy

Parameters	Incomplete abortion	Threatened abortion	Normal pregnancy
Incomplete abortion	-	<0.001*	<0.001*
Threatened abortion	<0.001*	-	0.01*
Normal pregnancy	<0.001*	0.01*	-

*p-value obtained by Tukey's test to determine the difference between two groups

Table 3: Difference of malondialdehyde level in incomplete abortion,
threatened abortion and normal pregnancy Malondialdehyde level

Parameters	Mean±SD (nmol mLG1)	95% CI	p-value*
Incomplete abortion	4.02±0.36	3.81-4.23	<0.001
Threatened abortion	3.46±0.39	3.24-3.68	
Normal pregnancy	2.83±0.21	2.71-2.96	

*p-values are obtained by ANOVA test, SD: Standard deviation, CI: Confidence interval

Table 4: Post hoc Test for the difference of the mean level of malondialdehyde in incomplete abortion,
threatened abortion and
normal pregnancy

Parameters	Incomplete abortion	Threatened abortion	Normal pregnancy
Incomplete abortion	-	<0.001*	<0.001*
Threatened abortion	<0.001*	-	<0.001*
Normal pregnancy	<0.001*	<0.001*	-

*p-value obtained by Tukey's test to determine the difference between two groups

As presented in Table 3, the mean level of MDA in incomplete abortion group was 4.02±0.36 nmol mLG1, the threatened abortion group was 3.46±0.39 nmol mLG1, whereas in normal pregnancy group was 2.83±0.21 nmol mLG1 with p = 0.00 by ANOVA test. To saw the difference between two group analysis continued with post hoc test. According to the Table 4, it was found that there were significant differences of MDA level between incomplete abortion with threatened abortion (p<0.001), incomplete abortion and normal pregnancy group (p<0.001), threatened abortion and normal pregnancy (p<0.001).

DISCUSSION

In this study, it was found that the mean of MDA level of incomplete abortion was higher than threatened abortion and normal pregnancy (p<0.05). Meanwhile, the mean level of CAT activity was lower in incomplete abortion than threatened abortion and normal pregnancy (p<0.05).

This study was accordance with a study conducted by Abdul-Barry et al.8 that found the serum MDA level was significantly higher in patients with a history of recurrent spontaneous abortion than women with a healthy pregnancy. Ozkaya et al.10 also reported that the spontaneous abortion before 8 weeks gestation was associated with higher serum of MDA than healthy pregnancy (66.4±13.7 vs. 40.3±16.1, p<0.01). Because MDA is a side product of lipid peroxidation, the increase in MDA level may reflect an overproduction of lipid peroxides and alter antioxidant defend mechanism.

Jauniaux et al.11 suggested the theory of how the free radical is higher in abortion than in normal pregnancy. They found that the intervillous O2 flow appeared much earlier in the abortion group so it can lead to excessive free radicals production that can cause abortion11.

The oxidative stress during pregnancy occurs in response to the fetoplacental energy demand. How the oxidative stress cause an abortion have been studied by other study. Many mechanisms have been proposed as the theory. Zhu et al.12, suggested three factors that has an impact on the pathogenesis of abortion. First, the ROS can cause

the lipid peroxidation injury in the embryo. Second, the increasing free radicals can change oxygen partial pressure in embryonic cells. And the last, increased of free radicals can lead to ischemia-reperfusion injury in the fetus12.

Actually, increased of antioxidants activity also found in early healthy pregnancy. These indicate that the oxidative stress actually also occurs in normal pregnancy but the sufficient antioxidants are defended the free radicals and prevented the damage6,13,14. In this study, the mean activity of CAT enzyme of incomplete abortion was lower than normal pregnancy and threatened abortion ($p<0.05$). It means that there were differences between the CAT enzyme activity in incomplete abortion, threatened abortion and normal pregnancy. It was suggested that the excess of free radicals and the decreased of antioxidants in this study were associated with the abortion.

Sugino et al.6 found that CAT total activity was decrease
and prostaglandin F2α synthesis was increase in decidua in cases of spontaneous abortion with vaginal bleeding. They proposed that the termination of pregnancy was caused by decreasing activity of CAT which stimulates the synthesis of prostaglandins6. This study was accordance with the study by Yigenoglu et al.15 that found the total antioxidant capacity is lower in *pregnant* women with history of spontaneous abortion than healthy pregnant women. It also accordance with a study conducted by Biri et al.16 that found the CAT activity was higher in abortion group than in control group (87.62±8.19 vs
44.47±3.79 IU mgG1).

CONCLUSION

Abortion group had lower antioxidant concentration and higher free radicals than normal pregnancy. There was an imbalance of free radical and antioxidant concentration in abortion.

SIGNIFICANT STATEMENT

The imbalance of free radical and antioxidant has been proposed to one of many theories that can cause abortion. This study tried to determine the comparison of antioxidant and free radical level by analyzed the catalase activity and malondialdehyde concentration in normal pregnancy and
abortion. Malondialdehyde (MDA) is a metabolite of lipid peroxides that used as an indicator of lipid peroxidation as a free radical. Meanwhile, catalase is one of antioxidant that works as free radicals binding by preventing lipid peroxidation in the cell membrane. The result of this study will determine the imbalance of free radicals and antioxidants in abortion and will help the medical field to develop the prevention of abortion.

Analyses of Nutrients and Body Mass Index as Risk Factor for Preeclampsia

Introduction

Preeclampsia is an important problem in obstetrics because it is still a *major* cause of maternal mortality compared to bleeding and infection. Preeclampsia leads to maternal and perinatal morbidity. Preeclampsia is also associated with high rates of preterm delivery, small for gestational ages, and perinatal death [1]. Little is known about the patho- genesis of preeclampsia. Many factors are identified as risk factors for preeclampsia including parity, multiple preg- nancies, age, family history of preeclampsia, obesity, his- tory of systemic disease, and nutrition.

Since preeclampsia is characterized by reduced perfu- sion of the placenta, oxidative stress, and endothelial dys- function, nutrition has long been hypothesized to have a role in the etiology of preeclampsia [2]. Oxidative stresses are proposed as the linkage between the two stages of preeclampsia. Nutrients can affect oxidative stress by increasing or decreasing free radicals or antioxidants or by providing substrate for the formation of free radicals. Several nutrients, particularly omega-3 (n - 3) fatty acids, antioxidants, and folic acid, have an important roles in modulating endothelial function. It has also been suggested that nutrients such as trace elements, fatty acids, and folic acid can contribute to insulin resistance, a risk factor for preeclampsia. In many studies, decrease in serum magne- sium levels has been considered as the cause of patho- genesis of preeclampsia. Minerals have an important

influence on the health of pregnant women and growing fetus. Among them, serum or placental zinc (Zn) concen- trations have been reported to be low in PE women. Fur- thermore, decreased levels of zinc, selenium, and copper have been observed in patients with preeclampsia [3]. In another side, the nutrients with antioxidants among high risk women showed a protective effect [4]. Folic acid has been hypothesized as a protective agent of preeclampsia.

Maternal obesity and insulin resistance are also believed to be important risk factors for the development of pla- cental endothelial dysfunction and preeclampsia. Preven- tion of preeclampsia has remained elusive, owing largely to their complex nature. Currently, maternal obesity in prepregnancy is one of the strongest modifiable risk fac- tors. Recent studies have shown a relation between obesity in prepregnancy and the risk of preeclampsia. The reason for obesity being associated with an increased risk of preeclampsia was explained by increased levels of serum triglycerides and very low-density lipoprotein particles in obese women. This lipid alterations have been suggested to promote oxidative stress caused by ischemia–reperfusion mechanism or activated neutrophils, which leads to endothelial cell dysfunction [1].

The hypothesis about nutritional status and body mass index (BMI) prepregnancy associated with preeclampsia has intrigued us to study the risk factor for preeclampsia in Dr. M. Djamil Hospital, Padang, Indonesia.

Methods

This was a case–control study at the Department of Obstetric and Gynecology in Dr. M. Djamil Hospital, Padang, Indonesia, between January and December 2013. Pregnant women after 20 weeks gestations were included. A total of 140 patients were enrolled in this study with 70 cases and 70 controls. Cases were those diagnosed with preeclampsia; meanwhile, controls were normotensive pregnant women without any other comorbidity.

After providing written informed consent, all subjects completed an interview for their nutritional status and prepregnancy BMI after delivery. The nutritional status was assessed by Food Frequency Questionnaire (FFQ) and then analyzed by Nutrisurvey Program. Calories, protein, fat, carbohydrates, calcium, phosphorus, zinc, sodium, potassium, magnesium, vitamin A, folic acid, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, vitamin C, and vitamin E were assessed. Maternal BMI was catego- rized into two groups: normal BMI and abnormal BMI. Normal BMI was defined as 18.5–24.9 kg/m2. The inde- pendent samples t test was used for nutritional status, and

Chi-square test was used for BMI. Odds ratio (OR) with

95% CI was calculated. A p value \0.05 was considered

statistically significant. For the nutrition variable, if the p value \0.25 then continued by logistic regression back- ward to assess the risk factor.

Results

Most of the subjects had normal weight, both in case and control groups. Table 1 shows that prevalence of abnormal BMI was more common in the preeclampsia group com- pared with those without preeclampsia 19 (27.1%) versus

12 (17.1%). BMI was not found as a significant risk factor in this study (p = 0.222).

As shown in Table 2, the mean level for most of the variables, except calories, fat, and vitamin B1, was lower in subjects with preeclampsia than those without preeclamp- sia. The difference for most of the subjects was also sta- tistically significant with p \ 0.05, except for vitamin B1 and vitamin B2. Table 3 shows the risk factors for preeclampsia obtained by logistic regression analyses. The nutrients that were significantly associated with increased risk of preeclampsia were deficiency of vitamin E, zinc, fat, calcium, and vitamin C. Excess of calories and carbohy- drate also significantly associated with increased risk of preeclampsia. Meanwhile, vitamin A and vitamin B1 were protective factors.

Discussion

The reason for obesity being associated with an increased risk of preeclampsia was explained by increased levels of serum triglycerides, very low-density lipoproteins, and formation of small low-density lipoprotein particles in obese women. This lipid profile was also found in women with preeclampsia. These lipid alterations have been suggested to promote oxidative stress, caused by ische- mia–reperfusion mechanism or activated neutrophils, which leads to endothelial cell dysfunction [1]. Moreover, dyslipidemia also can cause atherothrombosis and induce the aggregation of the thrombocytes than can lead to coagulopathy which is a characteristic of preeclampsia.

Obesity is accompanied by oxidative stress. The origin of oxidative stress is proposed to be secondary to increased free fatty acids and inflammation. It is also suggested that diet can contribute to oxidative stress. Obese individuals have lower blood concentrations of antioxidants. This could be due to reduced dietary intake of antioxidants, but increased consumption by reactive oxygen species is also possible [5].

In this study, we found no relationship between BMI with preeclampsia. As mentioned before, dyslipidemia is the important factor that can lead into preeclampsia. But our study did not assess profile lipid of the subjects. Fur- thermore, central obesity has a higher risk of preeclampsia. Central obesity is characterized by visceral fat. Visceral fat produces C-reactive protein (CRP), PAI-1, and leptin that contributes to oxidative stress. People with central obesity have a higher risk to get preeclampsia. In this study, we did not assess the central obesity of the subjects. Measures of body composition, including percent body fat, may very likely identify the obese woman at risk of preeclampsia more accurately.

Our study found that carbohydrate intake in preeclampsia group was *significantly* higher than the non-preeclampsia group. The subjects that have higher carbo- hydrate will have lower protein level. Meanwhile, protein is needed in the process of trophoblast invasion so the protein-energy malnutrition increases the risk of preeclampsia. We also found that intake of proteins in preeclampsia was significantly lower in the preeclampsia group.

Folic acid and vitamin B12 are also a protector factors against preeclampsia. Those micronutrients play an important role in suppressing the metabolism of homo- cysteine, whereas the excess of homocysteine was a cau- sative factor of endothelial damage and became one of the causes of preeclampsia. In addition, folic acid- supple- ments preconception enhance the placentation process and can prevent preeclampsia. Three earlier cohort studies assessed the effect of folic acid containing multivitamins (including folic acid) and gestational hypertension (in- cluding preeclampsia), and all showed a protective effect of folic acid supplementation on preeclampsia [2, 6, 7].

Table 1 Asssociation between body mass index and the risk of preeclampsia

BMI
Preeclampsia
%
Non-preeclampsia
%
p*
OR
CI 95%
Min.
Max.
Abnormal BMI
19
27.1
12
17.1

0.222
1.801
0.797
4.067
Normal BMI
51
72.9
58
82.9
70
100
70
100
BMI body mass index, OR odds ratio, CI confidence interval
* p value were obtained by Chi-square
Table 2 Differences of mean nutritions level in preeclampsia and non-preeclampsia group
Preeclampsia
Mean (95% CI)
Non-preeclampsia
Mean (95% CI)
p*
Calorie (Kcal)
1269.22 (745.2–2214)
1171.15 (649.1–2447.6)
0.048
Protein (g)
42.37 (23.03–95.42)
54.9 (24.2–200.6)
0.000
Fat (g)
26.8 (4.65–58.9)
32.4 (10.79–93.12)
0.009
KH (g)*
219.5 ± 56.8
163.49 ± 46.5
0.000
Calcium (mg)
234.7 (63.7–758.3)
9362.9 (63.7–922.4)
0.000
Phosphor (mg)
589.4 (296.5–1444.5)
714.6 (324.6–2353.4)
0.006
Fe (mg)
9.4 (2.84–23.9)
11.06 (2.84–35.6)
0.005
Zinc (mg)
4.8 (2.39–12.87)
5.5 (2.4–16.5)
0.005
Natrium (mg)

299.3 (76.9–970.24)
533.3 (105.9–1847.60)
0.000
Kalium (mg)*
595.4 ± 237.2
732.15 ± 367.8
0.010
Magnesium (mg)
181.1 (97.1–370.14)
210.0 (97.1–554.5)
0.010
Vitamin A (lg)
544.9 (174.9–1567.4)
763.9 (174.9–3354.5)
0.000
Folic acid (lg)
108.6 (56.8–421.6)
163.5 (56.83–497.9)
0.000
Vitamin B1 (mg)
0.7 (0.27–2.15)
0.65 (0.27–2.01)
0.085
Vitamin B2 (mg)
0.94 (0.25–2.45)
1.1 (0.25–2.7)
0.046
Niacin (mg)
4.3 (0.6–11.9)
5.8 (2.1–23.7)
0.000
Vitamin B6 (mg)
1.1 (0.66–2.14)
1.22 (0.67–3.8)
0.009
Vitamin B12 (mg)
1.1 (0.15–9.15)
2.8 (0.27–21.1)
0.000
Vitamin C (mg)
43.4 (8.4–136.8)
68.2 (19.2–262.7)
0.000
Vitamin E (mg TE)
1.9 (0.2–4.4)
3.6 (1.8–8.2)
0.000
CI confidence interval
* p value was obtained by independent samples t test

A recent large cohort study from Denmark also showed that regular use of folic acid in pregnancy was related to a reduced risk of preeclampsia among normal-weight women [8]. But in this study, we did not found the folic acid and vitamin B12 as a protective agent of preeclampsia. The different result maybe caused by supplementation that has been done generally. In other side, the no supplementation subject was rare so that selection bias/confounding become difficult to control.

The previous study showed that vitamin B6 deficiency is associated with cardiovascular disease. This is related to the function of B6 for the establishment of pyridoxal 5 phosphate which is an essential coenzyme in many meta- bolism processes. The coenzyme deficiency will lead to the diseases such as preeclampsia. Another micronutrients that play a role in the occurrence of preeclampsia is magne- sium. In a study, obtained serum magnesium levels in patients with preeclampsia were significantly lower than normal pregnancy. This indicates that magnesium as one of the predictors of preeclampsia. Magnesium also allegedly acted as a coenzyme intracellular and functions to maintain contraction and vascular tone [2].

Serum zinc, calcium, and magnesium levels have been compared between preeclamptic and healthy pregnant women in various studies. Some results showed that copper, zinc, and
calcium levels were significantly lower in preeclamptic patient, whereas magnesium concentrations showed no sig- nificant differences between *the* two groups [9]. Our study is consistent with that study, whereas we found zinc and calcium deficiency as a risk factor for preeclampsia (OR 99.46, 95% CI
1.37, 7219; OR 31, 95% CI 1.21, 841).

Our study found that vitamin E and A deficiency increase the risk of preeclampsia (OR 19.57, 95% CI
2.5, 151, OR 1768, 95% CI 10.22, 305,890). Antioxi-
dants are important in maintaining cellular integrity in *a* normal pregnancy by inhibiting peroxidation reaction and thus protecting enzymes, proteins, and cells from destruction by peroxides. Antioxidant defense mecha- nisms include cellular and extracellular enzymes such as free radical scavengers, including vitamin C and E, and metabolites such as bilirubin and uric acid. Vitamin C and E are antioxidants derived from the diet. Vitamin C scavenges free radicals in the aqueous phase, and the lipid-soluble vitamin E acts in vivo to prevent the for- mation of lipid peroxides and thus protect cell mem- branes. There is evidence of oxidative stress in women with established preeclampsia, including increased plasma concentration of 8-epi-prostaglandin F2a, lipid peroxides, and decreased *concentrations* of antioxidants such as vitamins C and E [10].

Table 3 Results of multivariate logistic regression analysis

	OR	p	95% confidence interval
Calorie	3.87	0.028	2.39 ± 6,268,293
Protein	0.049	0.650	0.27 ± 12,994
Fat	59.182	0.138	3.14 ± 500
KH	52.9	0.063	0.801 ± 3495
Calcium	109	0.119	0.29 ± 40,041
Fe	9.84	0.149	0.441 ± 219
Zinc	99.4	0.035	1.37 ± 7219
Natrium			

0.62
0.731
0.041 ± 9.37
Kalium
0.005
0.054
0.000 ± 1.08
Magnesium
0.015
0.528
0.000 ± 7321
Vitamin A
0.002
0.055
0.000 ± 1.14
Folic acid
42.9
0.193
0.15 ± 12,383
Vitamin B1
0.000
0.009
0.000 ± 0.014
Vitamin B2
3.75
0.550
0.049 ± 285
Niacin
2.39
0.068
0.571 ± 1E ? 007
Vitamin B6
0.299
0.591
0.004 ± 24.3
Vitamin B12
4.511
0.243
0.36 ± 56.4
Vitamin C
19.5
0.004
2.52 ± 151
Vitamin E
1.76
0.004
10.2 ± 30.5
OR odds ratio
Conclusions
Deficiency of vitamin E, zinc, fat, calcium, and vitamin C, and excess of calories and carbohydrate were associated with increased risk of preeclampsia.

Differences in Malondialdehyde and Catalase Activity Levels
Between Abortion and Normal Pregnancy

INTRODUCTION

Abortion is defined as a termination of a pregnancy before 20 weeks of gestation or when the fetal weight is less than 500 g. Clinically, the most frequent abortion encountered in the hospital is incomplete abortion. Patients usually come with bleeding and severe abdominal pain. The second one is threatened abortion. Threatened abortion is the most common complication in the first half of pregnancy and have incidence about 20-25%. Less than 30% of the women who experience threatened abortion will end in spontaneous abortion1.

There are many factors that associated with abortion, so it is hard to determine the exact mechanism. In spite of many possibilities, there is now a clear evidence that abortion is associated with placental oxidative stress. The abnormal placentation will lead to placental oxidative stress with resultant destructive effects on the syncytiotrophoblast. The theory has been proposed as a mechanism involved in the etiopathogenesis of abortion2.

The body has many antioxidant systems to defend the excessive Reactive Oxygen Species (ROS) production3. In a healthy body, ROS and antioxidant remain in balance. When the body has an excessive amount of ROS, Oxidative Stress (OS) then occurs. An increase in the expression of oxidative stress marker in the trophoblast was detected in abortion and this was speculated to be a cause of early pregnancy loss4.

Antioxidant system divided into enzymatic and nonenzymatic group. Catalase (CAT) is one of enzymatic antioxidant that can remove hydrogen peroxide (H2O2), prevents lipid peroxidation in the cell membrane and works as free radicals binding5. The CAT role in pregnancy is very important, in early pregnancy CAT plays a role for implantation by protecting the blastocysts from superoxide radicals in the endometrium. The CAT is also important for embryonic development and the maintenance of early pregnancy. The CAT level was found increased during pregnancy and low CAT activity in plasma or placenta found in cases of spontaneous abortion6. Another study also suggests that low antioxidant level increases the risk of spontaneous abortion7.

Free radicals are thus highly unstable molecules that have electrons available to react with a various organic substrate such as lipids, proteins and DNA8. Accumulation of lipid peroxides may cause not only tissue damage but also some biological events to accelerate the termination of pregnancy. It has been reported that ROS or lipid peroxide stimulate synthesis of PGF2α that causes uterine contraction6. Oxidative stress has been variably determined by many ways. The most reliable assessment of free radicals synthesis is using assays directly to detect the superoxide or other free radical. However, this technique is difficult and require specialized document. In another side, measurement of the products of oxidative modification product provides the most direct and possible assessment of oxidative stress. Malondialdehyde (MDA) is a metabolite of lipid peroxides that detectable in plasma and was used as an indicator of lipid peroxidation9. This study was carried out to *estimate* the mean *difference* of CAT and MDA in normal pregnancy, threatened abortion and incomplete abortion.

MATERIALS AND METHODS

This was an observational study with cross-sectional design on February-December, 2014. The population was all pregnant women before 20 weeks gestations were enrolled in the maternity ward of Dr. M. Djamil Hospital and Dr. Reksodiwiryo Hospital, Padang, Indonesia. Subjects were taken by consecutive sampling method. Cases were those diagnosed with threatened and incomplete abortion by a specialist doctor. Meanwhile, controls were normal pregnant women without any other comorbid.

Exclusion criteria were a mother with hydatidiform mole, uterus disorder, myoma, history of provocating and recurrent abortion. After providing informed, written consent, subject's blood was taken from antecubital vein for analyzed the CAT activity and MDA concentration with spectrophotometry method in Biomedical Laboratory of Medical Faculty of Andalas University.

The significant difference among the three groups was analyzed with ANOVA test. If $p<0.05$ was found, then the analysis continued with post hoc multiple comparison test to determined the differences between two group. Statistical analysis was conducted using a computer program.

RESULTS

During the study period, 42 blood samples were collected, consisting of 14 subjects with threatened abortion, 14 subjects with incomplete abortion and 14 subjects with normal pregnancy before 20 weeks gestations. Table 1 shows the activity of CAT enzyme in incomplete abortion group was 51.10±12.20 IU mgG1, the threatened abortion group was

66.46±9.11 IU mgG1, whereas normal pregnancy group was
78.26±8.88 IU mgG1. The analysis with statistical tests ANOVA found that p<0.001. To saw the specific difference between two subjects, the analysis continued by post hoc multiple comparisons test. As presented in Table 2, there were significant differences of CAT activity between incomplete abortion group and threatened abortion (p<0.001), incomplete abortion and normal pregnancy (p<0.001), threatened abortion and normal pregnancy (p = 0.01).

Table 1: Difference of catalase activity in incomplete abortion, threatened abortion and normal pregnancy Catalase activity

Parameters	Mean±SD (IU mgG1)	95% CI	p-value*
Incomplete abortion	51.10±12.20	44.06-58.15	<0.001
Threatened abortion	66.46±9.11	61.20-71.72	
Normal pregnancy	78.26±8.88	73.13-83.38	

*p-values are obtained by ANOVA test, SD: Standard deviation, CI: Confidence interval

Table 2: Post hoc test for the difference of the mean level of catalase activity in incomplete abortion, threatened abortion and normal pregnancy Incomplete Threatened Normal

Parameters	abortion	abortion	pregnancy
Incomplete abortion	-	<0.001*	<0.001*
Threatened abortion	<0.001*	-	0.01*
Normal pregnancy	<0.001*	0.01*	-

*p-value obtained by Tukey's test to determine the difference between two groups

Table 3: Difference of malondialdehyde level in incomplete abortion, threatened abortion and normal pregnancy Malondialdehyde level

Parameters	Mean±SD (nmol mLG1)	95% CI	p-value*
Incomplete abortion	4.02±0.36	3.81-4.23	<0.001
Threatened abortion	3.46±0.39	3.24-3.68	
Normal pregnancy	2.83±0.21	2.71-2.96	

*p-values are obtained by ANOVA test, SD: Standard deviation, CI: Confidence interval

Table 4: Post hoc Test for the difference of the mean *level* of malondialdehyde in incomplete abortion, threatened abortion and normal pregnancy Incomplete Threatened Normal

Parameters	abortion	abortion	pregnancy
Incomplete abortion	-	<0.001*	<0.001*
Threatened abortion	<0.001*	-	<0.001*
Normal pregnancy	<0.001*	<0.001*	-

*p-value obtained by Tukey's test to determine the difference between two groups

As presented in Table 3, the mean *level* of MDA in incomplete abortion group was 4.02±0.36 nmol mLG1, the threatened abortion group was 3.46±0.39 nmol mLG1, whereas in normal pregnancy group was 2.83±0.21 nmol mLG1 with p = 0.00 by ANOVA test. To saw the difference between two group analysis continued with post hoc test. According to the Table 4, it was found that there were significant differences of MDA level between incomplete abortion with threatened abortion (p<0.001), incomplete abortion and normal pregnancy group (p<0.001), threatened abortion and normal pregnancy (p<0.001).

DISCUSSION

In this study, it was found that the mean of MDA level of incomplete abortion was higher than threatened abortion and normal pregnancy (p<0.05). Meanwhile, the mean level of CAT activity was lower in incomplete abortion than threatened abortion and normal pregnancy (p<0.05).

This study was accordance with a study conducted by Abdul-Barry et al.8 that found the serum MDA level was *significantly* higher in patients with a history of recurrent spontaneous abortion than women with a healthy pregnancy. Ozkaya et al.10 also reported that the spontaneous abortion before 8 weeks gestation was associated with higher serum of MDA than healthy pregnancy (66.4±13.7 vs. 40.3±16.1, p<0.01). Because MDA is a side product of lipid peroxidation, the increase in MDA level may reflect an overproduction of lipid peroxides and alter antioxidant defend mechanism.

Jauniaux et al.11 suggested the theory of how the free radical is higher in abortion than in normal pregnancy. They found that the intervillous O2 flow appeared much earlier in the abortion group so it can lead to excessive free radicals production that can cause abortion11.

The oxidative stress during pregnancy occurs in response to the fetoplacental energy demand. How the oxidative stress cause an abortion have been studied by other study. Many mechanisms have been proposed as the theory. Zhu et al.12, suggested three factors that has an impact on the pathogenesis of abortion. First, the ROS can cause

the lipid peroxidation injury in the embryo. Second, the increasing free radicals can change oxygen partial pressure in embryonic cells. And the last, increased of free radicals can lead to ischemia-reperfusion injury in the fetus12.

Actually, increased of antioxidants activity also found in early healthy pregnancy. These indicate that the oxidative stress actually also occurs in normal pregnancy but the sufficient antioxidants are defended the free radicals and prevented the damage6,13,14. In this study, the mean activity of CAT enzyme of incomplete abortion was lower than normal pregnancy and threatened abortion ($p<0.05$). It means that there were differences between the CAT enzyme activity in incomplete abortion, threatened abortion and normal pregnancy. It was suggested that the excess of free radicals and the decreased of antioxidants in this study were associated with the abortion.

Sugino et al.6 found that CAT total activity was decrease
and prostaglandin F2α synthesis was increase in decidua in cases of spontaneous abortion with vaginal bleeding. They proposed that the termination of pregnancy was caused by decreasing activity of CAT which stimulates the synthesis of prostaglandins6. This study was accordance with the study by Yigenoglu et al.15 that found the total antioxidant capacity is lower in pregnant women with history of spontaneous abortion than healthy pregnant women. It also accordance with a study conducted by Biri et al.16 that found the CAT activity was higher in abortion group than in control group (87.62±8.19 vs
44.47±3.79 IU mgG1).

CONCLUSION

Abortion group had lower antioxidant concentration and higher free radicals than normal pregnancy. There was an imbalance of free radical and antioxidant concentration in abortion.

SIGNIFICANT STATEMENT

The imbalance of free radical and antioxidant has been proposed to one of many theories that can cause abortion. This study tried to determine the comparison of antioxidant and free radical level by analyzed the catalase activity and malondialdehyde concentration in normal pregnancy and
abortion. Malondialdehyde (MDA) is a metabolite of lipid peroxides that used as an indicator of lipid peroxidation as a free radical. Meanwhile, catalase is one of antioxidant that works as free radicals binding by preventing lipid peroxidation in the cell membrane. The result of this study will determine the imbalance of free radicals and antioxidants in abortion and will help the medical field to develop the prevention of abortion.

Research Article

Differences in Mean Levels of Maternal Resistin Serum between Early Onset Preeclampsia (EOPE) and Late Onset Preeclampsia (LOPE)

Research Article
Differences in Mean Levels of Maternal Resistin Serum between Early Onset Preeclampsia (EOPE) and Late Onset Preeclampsia (LOPE)

INTRODUCTION

Preeclampsia is one of the major causes of morbidity and mortality of the mother and fetus. World Health Organization (WHO) reported the number of deaths caused by preeclampsia by 16% in developing countries . Preeclampsia resulting 3-25 fold increased risk obstetric complications and is the cause of 30-40% of perinatal deaths in Indonesia [1] . The incidence of preeclampsia ranges between 5-10% of all pregnancies . Incidence of preeclampsia in the United States, Canada and Western Europe ranges between 2-5% of all pregnancies and higher, 4-18% in some developing countries in Africa [1] . The incidence of preeclampsia in Indonesia ranged between 3-10% [3] . In Dr.M. Djamil General Hospital in Padang during the [2] year 2011, the incidence of preeclampsia was 8.31%, on 2012 was 11.47% and on 2013 was 12.02%.

Preeclampsia is divided into early onset preeclampsia (EOPE) (<34 weeks) and late onset preeclampsia (LOPE) (>34 weeks) of pregnancy based on the onset of clinical manifestations of preeclampsia. The EOPE and LOPE has a different pathogenesis. The PEAD is *often* associated with impaired uteroplacental perfusion caused by disruption of trophoblast invasion, while LOPE is often associated with the presence of extrinsic and maternal factors .

Preeclampsia is a protean syndrome, in which multiple [4,5] organ systems can be affected compared to the others. Preeclampsia is mainly characterized by hypertension and proteinuria or may be associated with abnormalities in laboratory test results that renal function, hepatic or hemostasis after 20 weeks of pregnancy .

Analysis of risk factor for preeclampsia is needed to [1,6] mitigate the adverse effects of preeclampsia. One among of these risk factor is resistin which is associated with inflammation and insulin resistance, so that resistin was a

maternal risk factor that associated with LOPE
.
Resistin is an adipose tissue-specific secretory factor
7
(ADSF), a hormone secreted by adipose tissue that induces insulin resistance in muscle and liver. Resistin stimulated by inflammatory conditions that produce proinflammatory cytokines
.
Preeclampsia is associated with inflammation and insulin
7,8
resistance which is affected by resistin. Resistin is associated with late onset preeclampsia because it was a maternal factors, so there was an increasing of maternal resistin serum levels in late onset preeclampsia
.
Previous study reported an elevated of maternal resistin
7,8
serum levels associated with a systemic inflammatory response and insulin resistance which is both of them are
2
2
increased in preeclampsia compared to normal pregnancy
.
The other study showed the mean of Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and high sensitivity C-reactive protein (hs-CRP) was higher in late onset preeclampsia than early onset preeclampsia group.
From
various studies on the above it could be estimated that the inflammatory factor and insulin resistance are associated with higher levels of resistin more dominant in late onset preeclampsia group compared to early onset preeclampsia group.
The HOMA-IR could be used as an indicator of insulin resistance, but not for inflammatory states, contrary hs-CRP could be used as an indicator of inflammation, but not for insulin resistance. Resistin is expected to describe both of them, because it was associated with insulin resistance and inflammation
7
.

MATERIALS AND METHODS

This study is an analytical cross sectional study with 20 women of early onset preeclampsia (EOPE) and 20 women of late onset preeclampsia (LOPE) who met the inclusion criteria and there were no exclusion criteria. The samples were recruited in Dr.M. Djamil General Hospital, Padang from July-October, 2015.
The inclusions criteria were women who detected early onset preeclampsia or late onset preeclampsia in obstetrics emergency ward of Dr.M. Djamil General Hospital, Padang and willing to follow the research for taking blood samples.
The exclusions criteria were diabetes mellitus (random blood

sugar as >200 mg dLG
), had suffered coronary heart disease,
chronic kidney disease, chronic liver disease and obesity
(BMI before pregnancy >25 kg mG
1
).
Maternal resistin serum was examined by enzyme-linked
2
immunosorbent assay (ELISA) method in Biomedic Laboratory
in Medical Faculty of Andalas University. Statistical analysis
was conducted *by* using SPSS program 20th version. The data
distribution was normal with p>0.05. The differences mean
levels of maternal resistin serum was analyzed by using
independent t-test.

RESULTS

Characteristics of research subjects: Forty patients of
research subjects consisted 20 patients of early onset
preeclampsia (EOPE) and 20 patients of late onset
preeclampsia (LOPE). Mean levels of maternal age in EOPE
group is 34.4±5.144 years, whereas in LOPE group is
32.9±6.324 years with p = 0.416. According to maternal age
9-12

Table 1: Characteristics of research subjects between early onset preeclampsia and late onset preeclampsia

Characteristics EOPE (n = 20) LOPE (n = 20) Total (%) p
Maternal age group
<20 years 0 (0%) 0 (0%) 0
20-35 years 8 (40%) 10 (50%) 45 0.537
>35 years 12 (60%) 10 (50%) 55
Maternal age (0±SD) years 34.4±5.144 31.9±6.314 0.416
Gravidity group
Primigravid 6 (30%) 7 (35%) 32.5 0.744
Miltigravid 14 (70%) 13 (65%) 67.5
Gravidity (0±SD) 2.4±1.314 2.65±1.461 0.573
BMI group
Underweight 0 (0%) 0 (0%) 0
Normoweight 14 (70%) 12 (60%) 65 0.520
Overweight 6 (30%) 8 (40%) 35
BMI (0±SD) 22.55±1.595 22.59±1.812 0.946

Table 2: Mean levels of maternal resistin serum in early onset preeclampsia and
late onset preeclampsia

EOPE LOPE
---------- ----------Variable
Mean SD Mean SD
Maternal resistin serum (ng mLG
1
) 2.526 1.603 8.891 6.219

Table 3: Mean levels difference of maternal resistin serum in early onset
preeclampsia and late onset preeclampsia

Variable Mean difference CI (95%) p
Maternal resistin serum (ng mLG
1
) 6.365 3.3835-9.3475 0.000

groups, study of subjects in early onset preeclampsia
consists 8 patients (40%) in group of maternal age

20-35 years old, 12 patients (60%) in group of maternal age >35 years old, none in group of maternal age <20 years old. Research subjects of late onset preeclampsia consists of 1 0 patients (50%) in group of maternal age 20-35 years old, 10 patients (50%) in group of maternal age >35 years old, none in group of maternal age <20 years old.

Mean levels of gravidity in early onset preeclampsia (EOPE) group is 2.4±1.314, whereas in late onset preeclampsia (LOPE) group is 2.65±1.461 with p = 0.573. According to gravidity groups, study of subjects in early onset preeclampsia consists 6 patients (30%) in group of primigravida, 14 patients (70%) in group of multigravida. Research subjects of l ate onset preecl ampsi a c onsi sts of 7 patients (35%) in group of primigravida, 13 patients (65%).

Mean levels of Body Mass Index (BMI) in early onset preeclampsia (EOPE) group is 22.551±1.595, whereas in late onset preeclampsia (LOPE) group is 22.588±1.4812 kg mG with p = 0.946. According to BMI groups, study of subjects in EOPE consists 14 patients (70%) in group of normal weight, 6 patients (30%) in group of overweight, none in group BMI underweight. Research subjects of late onset preeclampsia consists 12 patients (60%) in group of normal weight, 8 patients (40%) in group of overweight, none in group BMI underweight.

2

3

There were no statistically significant differences regarding maternal age, group of maternal age, gravidity, group of gravidity, BMI and group of BMI characteristic between *those* two groups with p>0.05 showed in Table 1.

Mean levels of maternal resistin serum in early onset preeclampsia and late onset preeclampsia: Mean levels of maternal resistin serum was higher in late onset preeclampsia vs

2.526±1.603 ng dLG

than early onset preeclampsia (8.891±6.219 ng mLG

1

), showed in Table 2.

Differences in mean levels of maternal resistin serum between late onset preeclampsia and early onset preeclampsia: The data distribution was normal with Kolmogorov Smirnov test. Analysis was performed with independent t-test. Table 3 showed the differences in mean levels of maternal resistin serum between early onset preeclampsia and late onset preeclampsia as 6.365 ng mLG with Confidence Interval (CI) 95% 3.384-9.347. There is a high significant differences with p<0.001.

DISCUSSION

The mean levels of maternal serum resistin in early onset preeclampsia (EOPE) group was 2.526±1.603 ng mLG whereas in late onset preeclampsia (LOPE) group was 8.891±6.219 ng mLG

(Table 2), with a mean difference

6.365 ng dLG

1
1
, 95% CI 3.384-9.347. Statistical test showed p<0.001, that the mean *levels* of maternal serum resistin significantly was higher in LOPE than EOPE (Table 3). Resistin is a hormone secreted from adipose tissue that induces insulin resistance in muscle and liver. Resistin stimulated by inflammatory conditions that produce proinflammatory cytokines. Resistin contribute to the inflammatory disorders such as coronary heart disease,
1
1
1
chronic kidney disease and liver cirrhosis as well as insulin resistance in diabetes mellitus through the activation of proinflammatory cytokines varied which depending on the organs affected
.
Insulin resistance and inflammation were the condition
7,8
associated to preeclampsia. This factor related to the role of resistin through the release of proinflammatory cytokines. The relationship between resistin and preeclampsia is connected by those which is a maternal factor in preeclampsia. Therefore, resistin is associated to late onset preeclampsia, so the levels of maternal resistin serum was increased in late onset preeclampsia
.
Inflammation and insulin resistance have been
7,8
investigated, that the comparasion between HOMA-IR and hs-CRP in EOPE and LOPE. This study reported the results that mean levels of HOMA-IR and hs-CRP were higher in LOPE compared to EOPE (HOMA IR: 4.86±5.50 vs 3.99±5.97 and hsCRP: 123.08±38.67 vs 26.54±34.7 mg LG
). This study suggested that the inflammatory factor and insulin resistance were more dominant in LOPE compared to EOPE.
1
A s i mi l ar r es ul t s w as r epor t ed i n whi c h t he mean l evel s of maternal resistin serum was higher in preeclampsia compared to normal pregnancy related to an increase in insulin resistance and the response systemic inflammation that happened in preeclampsia particularly late onset preeclampsia
.
A cross sectional study in which two groups of pregnant
9-12
women with preeclampsia (n = 15) and normal pregnancy (n = 23) demonstrated th mean levels of maternal resistin serum was higher in preeclampsia (5.68±0.41 ng mLG
)
compared to normal pregnancy (4. 65±0. 32 ng mLG
),

p = 0.028. The mean levels of maternal resistin serum related to the mean of HOMA-IR and proinflammatory cytokines that were elevated in preeclampsia compared to normal pregnancy. T he mean of H OMA-I R (2. 5±0. 8 vs 1. 4±0. 1), IL-6 (6.34±1.02 vs 2.80±0.31) and TNF-" (1.89±0.18 vs 1.23±0.10)

.

Another cross-sectional study on two groups of 9 pregnant women, which were preeclampsia (n = 29) and normal pregnancy (n = 30), found that mean levels of maternal resistin serum was higher in preeclampsia 61.98±32.26 ng dLG , compared to normal pregnancy 38.06±31.26 ng dLG 1 1 , p = 0.013. Insulin resistance is thought related the increasing the levels of maternal resistin serum, because the mean of HOMA-IR was higher in preeclampsia compared to normal pregnancy (4:44±4:02 vs 3.99±2.82)

.

A cross sectional study was conducted on two groups of pregnant women, which are preeclampsia (n = 50) and normal pregnancy (n = 50). The median levels of maternal resistin 1 1 10 4 serum were higher in preeclampsia 61 ng mLG compared to normal pregnancy 25.5 ng mLG 1 1 , p = 0.033. Insulin resistance is thought related the increasing the levels of maternal resistin serum, because the median of HOMA-IR was higher in preeclampsia compared to normal pregnancy (4.7 vs 3.6)

.

A cross sectional study was conducted on two groups of pregnant women, which are preeclampsia (n = 16) and normal pregnancy (n = 22). The mean levels of maternal resistin serum was higher in preeclampsia (12.06±0.973 ng mLG) compared t o n ormal p regnancy (7. 35±1. 195 ng mLG), p = 0.041. Inflammatory factors is thought related the increasing the levels of maternal resistin serum in preeclampsia. There was an increasing mean levels of TNF" in preeclampsia compared to normal pregnancy (15.23±0.674 vs 12.84±0.348 ng mLG 1 , p = 0.021) . Based on

that description, there was a correlation between previous studies and this study
9-12
12
.
The mean levels of HOMA-IR and hs-CRP were higher in late onset preeclampsia than early onset preeclampsia, so according to those studies above, the insulin resistance and inflammation that were higher in late onset preeclampsia than early onset preeclampsia related to the levels of maternal resistin serum that was higher in late onset preeclampsia than early onset preeclampsia. It was appropriate with the results of this research that the mean levels of maternal resistin serum in late onset preeclampsia was *significantly* higher than early onset preeclampsia with $p<0.001$.
CONCLUSION
The mean levels of maternal resistin serum was higher significantly in late onset preeclampsia (LOPE) compared to early onset preeclampsia (EOPE).
11
1
1

Differences in brain-derived neurotrophic factor between neonates born to mothers with normal and low ferritin

INTRODUCTION

Nutrition plays a role in the intellectual development of a child during intrauterine life. The most common nutritional deficiency found in *pregnant* women is iron defi- ciency.1,2 Pregnancy is a condition that increases the iron requirement to meet fetal and placental needs. Insuffi- cient iron stores before pregnancy and inadequate iron intake during pregnancy can lead to iron deficiency ane- mia.3 The iron deficiency can be examined by assessing the serum ferritin that directly correlates with the total amount of iron stored in the body. Iron deficiency anemia is usually found when the serum ferritin is less than 12 µg/dL.4,5

A study by Milman et al (1991) suggested that neonates born to mothers receiving iron supplementation during pregnancy had higher serum ferritin than neonates born to mothers receiving placebo.6 Consistent with the previous study, Gaspar et al (1993) and Perez et al (2005) reported that neonates born to mothers with low serum ferritin tend to have low serum ferritin as well.7,8 Shao et al (2012) also found that there was a positive correlation between maternal serum ferritin and iron reserve in term neonates.9

Iron deficiency is associated with decreased intellectual or cognitive function. The brain region that serves as a center of memory, learning, and behavior is the hippo- campus. Iron is essential for the hippocampus to undertake dendritogenesis, synaptogenesis, neurogenesis, and synthesis of neurotrophic factors that facilitate brain growth and development commencing in intrauterine life. The main neurotrophic factor in the hippocampus for learning, memory, and behavior is brain-derived neu- rotrophic factor (BDNF). BDNF is a family of neurotrophins that plays an important role in neuronal cell plas- ticity, oligodendrocyte growth modulation, myelin formation, dendritogenesis, augmentation of glutamatergic synapse transmission, and mitigation of the gamma-amino butyric acid (GABA) inhibitory signal.10,11

Animal studies have found that iron deficiency in early life leads to epigenetic changes which alter the structure of chromatin and gene expression of BDNF, thus causing a decrease in neuronal differentiation in the hippocampus. This is also associated with abnormalities of behavior and cognitive function.12 Acute iron deficiency that occurs in late pregnancy, delivery, and postpartum periods may result in reduction of BDNF and mitochondrial dysfunc- tion. This can lead to impairment of neuron structure, neuron electrophysiology, and behavioral development. If the iron deficiency still persists into adulthood, it may also contribute to learning and memory problems.5,13

The susceptibility of pregnant women to iron deficien- cy anemia, the positive correlation between maternal and neonatal ferritin, and the *negative* effect of iron deficien- cy on brain function have encouraged us to study the dif- ferences in BDNF among term newborns from mothers with normal and low ferritin.

METHODS

This was an observational study with a cross-sectional design. It was conducted from August 2015 to February 2016 and approved by the Medical Research Ethics Committee of the Dr. M. Djamil Hospital (Project Num- ber PE.07.2016). Term pregnant patients with normal leukocyte counts and willing to participate were include. The exclusion criteria were any evidence of infectious disease, vascular disease, kidney disease, diabetes melli- tus, or vaginal bleeding. Eligible patients were identified through the cooperation of three participating medical institutions located in Padang, West Sumatra, Indonesia, namely, Yarsi hospital, BMC hospital, and Hardi clinic. Those who agreed to participate in the study gave written informed consent and had their blood checked for serum

ferritin. A total of 40 pregnant women participated, 20 with normal serum ferritin (>12 ng/mL) and 20 with low serum ferritin (<12 ng/mL).

All participants were followed until delivery and the cord-blood of their newborns was examined immediately t after birth for BDNF using an enzyme-linked immuno- sorbent assay (ELISA) method available in the Biomedical Laboratory of the Medical faculty of Andalas Univer- sity. Independent sample t-tests were used to assess sig- nificant difference (SPSS program (22.0 version).

RESULTS

Maternal characteristics are shown in Table 1.There were no differences in age, parity, Body Mass Index (BMI) or leukocyte counts between normal and low ferritin groups ($p>0.05$). However, there was a significant difference in hemoglobin between the two groups ($p<0.05$).

Neonatal characteristics are shown in Table 2. The dif- ferences in birth weight, body length, and head circumference among newborns from mothers with normal and low ferritin were not statistically significant ($p>0.05$). There was a significant difference in neonatal BDNF be- tween the normal and low maternal ferritin groups ($p<0.05$) (Figure 1).

DISCUSSION

As shown in Table 1, we found that those with low ferri- tin had a lower hemoglobin than those with normal ferri- tin ($p<0.05$). This is consistent with a study by Milman et al (1991) which was a placebo controlled study of 207 healthy Danish women after 16 week gestation. They reported that the placebo-treated group had a lower he-

Table 1. The maternal characteristics by maternal ferritin

Characteristics	Normal ferritin (≥12 ng/mL) N=20	Low ferritin (<12 ng/mL) N=20	p value*
Age (years)†	31.1±5.42	29.9±5.29	0.268
Parity‡			
Primipara	10 (50%)	13 (65%)	0.344
Multipara	10 (50%)	7 (35%)	
BMI (kg/m2)†	23.0±2.13	23.1±4.34	0.355
Hb (gr/L)†	109±6.9	101±12.3	0.025
Leukocytes (109/L)†	9.7±1.7	10.3±2.0	0.319

BMI: body mass index; Hb: haemoglobin.
†Data are reported as mean±SD.
‡Data are reported as percentage.
*p values are obtained by independent t-test.

Table 2. The neonatal characteristics by maternal ferritin

Sex†	Normal ferritin (≥12 ng/mL) N=20	Low ferritin (<12 ng/mL) N=20	Mean differenece * (95% CI)	p value
Men	9 (45%)	15 (75%)		
Women	11 (55%)	5 (25%)		
Birth weight (kg)‡	3.16±0.31	3.29±0.34	124 (87.1-336)	0.241
Body length (cm)‡	48.8±2.09	48.4±1.73	0.40 (0.33-1.63)	0.319
Head circumference (cm)‡	34.4±0.59	34.3±0.470	0.1 (0.04-0.44)	0.667
BDNF serum (ng/mL)‡	3.81±1.37	2.78±1.19	1.03 (0.21-1.86)	0.015

BDNF: brain-derived neurotrophic factor; CI: confidence interval.
†Data are reported as percentage.
‡Data are reported as mean±SD.
*p values are obtained by independent t-test.

Figure 1. Differences in mean level of Brain Derived Neurotrphic Factor (BDNF) between the newborns babies

from mother with normal
ferritin levels and low ferritin levels.
moglobin than the iron-treated group (p<0.001). Sao et al (2012) conducted a study in Southeastern China of *3702* pregnant women after 37 week gestation. They found that maternal anemia (Hb <11 g/dL) was present in 27.5% women and associated with low maternal serum ferritin (<20 ng/mL) in 86.9% women.6,9
Iron plays an important role in the synthesis of neuro- transmitters such as serotonin, norepinephrine, and dopamine that are essential for the synthesis of BDNF.11
Acute iron deficiency that occurs in late pregnancy and postpartum will interfere with neuronal structure and electrophysiology. As indicated above, if the iron defi- ciency persists into adulthood, learning and memory disorders may supervene.5,12 The associated decrease in BDNF expression compromises neuron differentiation in several brain areas. This underscores the importance of iron homeostasis from the commencement of central nervous system (CNS) intrauterine development.12
The observed association of lower neonatal BDNF with a low maternal ferritin is biologically plausible. Serum ferritin has a direct correlation with the total amount of iron stored in the body. Maternal iron deficiency during pregnancy has a negative effect on fetal iron homeostasis. Iron transfer from mother to fetus is a regulated process involving iron status in the maternal circulation, its transport across the placenta and subsequent transfer into the fetal circulation. The resulting iron deficiency in neo- nates may decrease the expression and function BDNF in specific areas of the brain and be reflected in a low plas- ma BDNF. Similar to the present findings, Gaspar found that cord-blood ferritin in neonates born to mothers with low serum ferritin was lower *than* in those born to moth- ers with normal serum ferritin.7 This is also in accordance with studies conducted by Perez in 2005 and Shao in
2012 who found that positive correlations between mater- nal serum ferritin and the iron reserve of term neonates.8,9
Given the role of BDNF in learning and memory, it is imperative that all pregnant women should be given iron replete not only to prevent any iron deficiency-related morbidity, but also to provide the mother the optimal opportunity of having intelligent offspring. The World
Health Organization (WHO) recommends a daily sup- plement of 30 to 60 mg of elemental iron as part of the antenatal care in settings where anemia in pregnant wom- en is a public health problem. A higher dose may be nec- essary if the pregnant women is diagnosed with anemia and until her hemoglobin concentration becomes nor- mal.14
A limitation of our study is the absence of neonatal fer- ritin data so that the direct relationship between maternal and neonatal ferritin could not be determined. Whether the decreased neonatal BDNF was attributable to decreased neonatal ferritin could not be determined.

Analyses of Nutrients and Body Mass Index as Risk Factor for Preeclampsia

Introduction

Preeclampsia is an important problem in obstetrics because it is still a major cause of maternal mortality compared to bleeding and infection. Preeclampsia leads to maternal and perinatal morbidity. Preeclampsia is also associated with high rates of preterm delivery, small for gestational ages, and perinatal death [1]. Little is known about the patho- genesis of preeclampsia. Many factors are identified as risk factors for preeclampsia including parity, multiple preg- nancies, age, family history of preeclampsia, obesity, his- tory of systemic disease, and nutrition.

Since preeclampsia is characterized by reduced perfu- sion of the placenta, oxidative stress, and endothelial dys- function, nutrition has long been hypothesized to have a role in the etiology of preeclampsia [2]. Oxidative stresses are proposed as the linkage between the two stages of preeclampsia. Nutrients can affect oxidative stress by increasing or decreasing free radicals or antioxidants or by providing substrate for the formation of free radicals. Several nutrients, particularly omega-3 (n - 3) fatty acids, antioxidants, and folic acid, have an important roles in modulating endothelial function. It has also been suggested that nutrients such as trace elements, fatty acids, and folic acid can contribute to insulin resistance, a risk factor for preeclampsia. In many studies, decrease in serum magne- sium levels has been considered as the cause of patho- genesis of preeclampsia. Minerals have an important

influence on the health of pregnant women and growing fetus. Among them, serum or placental zinc (Zn) concen- trations have been reported to be low in PE women. Fur- thermore, decreased levels of zinc, selenium, and copper have been observed in patients with preeclampsia [3]. In another side, the nutrients with antioxidants among high risk women showed a protective effect [4]. Folic acid has been hypothesized as a protective agent of preeclampsia.

Maternal obesity and insulin resistance are also believed to be important risk factors for the development of pla- cental endothelial dysfunction and preeclampsia. Preven- tion of preeclampsia has remained elusive, owing largely to their complex nature. Currently, maternal obesity in prepregnancy is one of the strongest modifiable risk fac- tors. Recent studies have shown a relation between obesity in prepregnancy and the risk of preeclampsia. The reason for obesity being associated with an increased risk of preeclampsia was explained by increased levels of serum triglycerides and very low-density lipoprotein particles in obese women. This lipid alterations have been suggested to promote oxidative stress caused by ischemia–reperfusion mechanism or activated neutrophils, which leads to endothelial cell dysfunction [1].

The hypothesis about nutritional status and body mass index (BMI) prepregnancy associated with preeclampsia has intrigued us to study the risk factor for preeclampsia in Dr. M. Djamil Hospital, Padang, Indonesia.

Methods

This was a case–control study at the Department of Obstetric and Gynecology in Dr. M. Djamil Hospital, Padang, Indonesia, between January and December 2013. Pregnant women after 20 weeks gestations were included. A total of 140 patients were enrolled in this study with 70 cases and 70 controls. Cases were those diagnosed with preeclampsia; meanwhile, controls were normotensive pregnant women without any other comorbidity.

After providing written informed consent, all subjects completed an interview for their nutritional status and prepregnancy BMI after delivery. The nutritional status was assessed by Food Frequency Questionnaire (FFQ) and then analyzed by Nutrisurvey Program. Calories, protein, fat, carbohydrates, calcium, phosphorus, zinc, sodium, potassium, magnesium, vitamin A, folic acid, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, vitamin C, and vitamin E were assessed. Maternal BMI was catego- rized into two groups: normal BMI and abnormal BMI. Normal BMI was defined as 18.5–24.9 kg/m2. The inde- pendent samples t test was used for nutritional status, and

Chi-square test was used for BMI. Odds ratio (OR) with

95% CI was calculated. A p value \0.05 was considered

statistically significant. For the nutrition variable, if the p value \0.25 then continued by logistic regression back- ward to assess the risk factor.

Results

Most of the subjects had normal weight, both in case and control groups. Table 1 shows that prevalence of abnormal BMI was more common in the preeclampsia group com- pared with those without preeclampsia 19 (27.1%) versus

12 (17.1%). BMI was not found as a significant risk factor in this study (p = 0.222).

As shown in Table 2, the mean level for most of the variables, except calories, fat, and vitamin B1, was lower in subjects with preeclampsia than those without preeclamp- sia. The difference for most of the subjects was also sta- tistically significant with p \ 0.05, except for vitamin B1 and vitamin B2. Table 3 shows the risk factors for preeclampsia obtained by logistic regression analyses. The nutrients that were significantly associated with increased risk of preeclampsia were deficiency of vitamin E, zinc, fat, calcium, and vitamin C. Excess of calories and carbohy- drate also significantly associated with increased risk of preeclampsia. Meanwhile, vitamin A and vitamin B1 were protective factors.

Discussion

The reason for obesity being associated with an increased risk of preeclampsia was explained by increased levels of serum triglycerides, very low-density lipoproteins, and formation of small low-density lipoprotein particles in obese women. This lipid profile was also found in women with preeclampsia. These lipid alterations have been suggested to promote oxidative stress, caused by ische- mia–reperfusion mechanism or activated neutrophils, which leads to endothelial cell dysfunction [1]. Moreover, dyslipidemia also can cause atherothrombosis and induce the aggregation of the thrombocytes than can lead to coagulopathy which is a characteristic of preeclampsia.

Obesity is accompanied by oxidative stress. The origin of oxidative stress is proposed to be secondary to increased free fatty acids and inflammation. It is also suggested that diet can contribute to oxidative stress. Obese individuals have lower blood concentrations of antioxidants. This could be due to reduced dietary intake of antioxidants, but increased consumption by reactive oxygen species is also possible [5].

In this study, we found no relationship between BMI with preeclampsia. As mentioned before, dyslipidemia is the important factor that can lead into preeclampsia. But our study did not assess profile lipid of the subjects. Fur- thermore, central obesity has a higher risk of preeclampsia. Central obesity is characterized by visceral fat. Visceral fat produces C-reactive protein (CRP), PAI-1, and leptin that contributes to oxidative stress. People with central obesity have a higher risk to get preeclampsia. In this study, we did not assess the central obesity of the subjects. Measures of body composition, including percent body fat, may very likely identify the obese woman at risk of preeclampsia more accurately.

Our study found that carbohydrate intake in preeclampsia group was significantly higher than the non-preeclampsia group. The subjects that have higher carbo- hydrate will have lower protein level. Meanwhile, protein is needed in the process of trophoblast invasion so the protein-energy malnutrition increases the risk of preeclampsia. We also found that intake of proteins in preeclampsia was significantly lower in the preeclampsia group.

Folic acid and vitamin B12 are also a protector factors against preeclampsia. Those micronutrients play an important role in suppressing the metabolism of homo- cysteine, whereas the excess of homocysteine was a cau- sative factor of endothelial damage and became one of the causes of preeclampsia. In addition, folic acid-supple- ments preconception enhance the placentation process and can prevent preeclampsia. Three earlier cohort studies assessed the effect of folic acid containing multivitamins (including folic acid) and gestational hypertension (in- cluding preeclampsia), and all showed a protective effect of folic acid supplementation on preeclampsia [2, 6, 7].

Table 1 Asssociation between body mass index and the risk of preeclampsia

BMI
Preeclampsia
%
Non-preeclampsia
%
p*
OR
CI 95%
Min.
Max.
Abnormal BMI
19
27.1
12
17.1

0.222
1.801
0.797
4.067
Normal BMI
51
72.9
58
82.9
70
100
70
100
BMI body mass index, OR odds ratio, CI confidence interval
* p value were obtained by Chi-square
Table 2 Differences of mean nutritions level in preeclampsia and non-preeclampsia group
Preeclampsia
Mean (95% CI)
Non-preeclampsia
Mean (95% CI)
p*
Calorie (Kcal)
1269.22 (745.2–2214)
1171.15 (649.1–2447.6)
0.048
Protein (g)
42.37 (23.03–95.42)
54.9 (24.2–200.6)
0.000
Fat (g)
26.8 (4.65–58.9)
32.4 (10.79–93.12)
0.009
KH (g)*
219.5 ± 56.8
163.49 ± 46.5
0.000
Calcium (mg)
234.7 (63.7–758.3)
9362.9 (63.7–922.4)
0.000
Phosphor (mg)
589.4 (296.5–1444.5)
714.6 (324.6–2353.4)
0.006
Fe (mg)
9.4 (2.84–23.9)
11.06 (2.84–35.6)
0.005
Zinc (mg)
4.8 (2.39–12.87)
5.5 (2.4–16.5)
0.005
Natrium (mg)

299.3 (76.9–970.24)
533.3 (105.9–1847.60)
0.000
Kalium (mg)*
595.4 ± 237.2
732.15 ± 367.8
0.010
Magnesium (mg)
181.1 (97.1–370.14)
210.0 (97.1–554.5)
0.010
Vitamin A (lg)
544.9 (174.9–1567.4)
763.9 (174.9–3354.5)
0.000
Folic acid (lg)
108.6 (56.8–421.6)
163.5 (56.83–497.9)
0.000
Vitamin B1 (mg)
0.7 (0.27–2.15)
0.65 (0.27–2.01)
0.085
Vitamin B2 (mg)
0.94 (0.25–2.45)
1.1 (0.25–2.7)
0.046
Niacin (mg)
4.3 (0.6–11.9)
5.8 (2.1–23.7)
0.000
Vitamin B6 (mg)
1.1 (0.66–2.14)
1.22 (0.67–3.8)
0.009
Vitamin B12 (mg)
1.1 (0.15–9.15)
2.8 (0.27–21.1)
0.000
Vitamin C (mg)
43.4 (8.4–136.8)
68.2 (19.2–262.7)
0.000
Vitamin E (mg TE)
1.9 (0.2–4.4)
3.6 (1.8–8.2)
0.000
CI confidence interval
* p value was obtained by independent samples t test

A recent large cohort study from Denmark also showed that regular use of folic acid in pregnancy was related to a reduced risk of preeclampsia among normal-weight women [8]. But in this study, we did not found the folic acid and vitamin B12 as a protective agent of preeclampsia. The different result maybe caused by supplementation that has been done generally. In other side, the no supplementation subject was rare so that selection bias/confounding become difficult to control.

The previous study showed that vitamin B6 deficiency is associated with cardiovascular disease. This is related to the function of B6 for the establishment of pyridoxal 5 phosphate which is an essential coenzyme in many meta- bolism processes. The coenzyme deficiency will lead to the diseases such as preeclampsia. Another micronutrients that play a role in the occurrence of preeclampsia is magne- sium. In a study, obtained serum magnesium levels in patients with preeclampsia were significantly lower than normal pregnancy. This indicates that magnesium as one of the predictors of preeclampsia. Magnesium also allegedly acted as a coenzyme intracellular and functions to maintain contraction and vascular tone [2].

Serum zinc, calcium, and magnesium levels have been compared between preeclamptic and healthy pregnant women in various studies. Some results showed that copper, zinc, and
calcium levels were significantly lower in preeclamptic patient, whereas magnesium concentrations showed no sig- nificant differences between the two groups [9]. Our study is consistent with that study, whereas we found zinc and calcium deficiency as a risk factor for preeclampsia (OR 99.46, 95% CI
1.37, 7219; OR 31, 95% CI 1.21, 841).

Our study found that vitamin E and A deficiency increase the risk of preeclampsia (OR 19.57, 95% CI
2.5, 151, OR 1768, 95% CI 10.22, 305,890). Antioxi-
dants are important in maintaining cellular integrity in a normal pregnancy by inhibiting peroxidation reaction and thus protecting enzymes, proteins, and cells from destruction by peroxides. Antioxidant defense mecha- nisms include cellular and extracellular enzymes such as free radical scavengers, including vitamin C and E, and metabolites such as bilirubin and uric acid. Vitamin C and E are antioxidants derived from the diet. Vitamin C scavenges free radicals in the aqueous phase, and the lipid-soluble vitamin E acts in vivo to prevent the for- mation of lipid peroxides and thus protect cell mem- branes. There is evidence of oxidative stress in women with established preeclampsia, including increased plasma concentration of 8-epi-prostaglandin F2a, lipid peroxides, and decreased concentrations of antioxidants such as vitamins C and E [10].

Table 3 Results of multivariate logistic regression analysis

	OR	p	95% confidence interval
Calorie	3.87	0.028	2.39 ± 6,268,293
Protein	0.049	0.650	0.27 ± 12,994
Fat	59.182	0.138	3.14 ± 500
KH	52.9	0.063	0.801 ± 3495
Calcium	109	0.119	0.29 ± 40,041
Fe	9.84	0.149	0.441 ± 219
Zinc	99.4	0.035	1.37 ± 7219
Natrium			

0.62
0.731
0.041 ± 9.37
Kalium
0.005
0.054
0.000 ± 1.08
Magnesium
0.015
0.528
0.000 ± 7321
Vitamin A
0.002
0.055
0.000 ± 1.14
Folic acid
42.9
0.193
0.15 ± 12,383
Vitamin B1
0.000
0.009
0.000 ± 0.014
Vitamin B2
3.75
0.550
0.049 ± 285
Niacin
2.39
0.068
0.571 ± 1E ? 007
Vitamin B6
0.299
0.591
0.004 ± 24.3
Vitamin B12
4.511
0.243
0.36 ± 56.4
Vitamin C
19.5
0.004
2.52 ± 151
Vitamin E
1.76
0.004
10.2 ± 30.5
OR odds ratio
Conclusions
Deficiency of vitamin E, zinc, fat, calcium, and vitamin C, and excess of calories and carbohydrate were associated with increased risk of preeclampsia.

Differences in brain-derived neurotrophic factor between neonates born to mothers with normal and low ferritin

INTRODUCTION

Nutrition plays a role in the intellectual development of a child during intrauterine life. The most common nutri- tional deficiency found in pregnant women is iron defi- ciency.1,2 Pregnancy is a condition that increases the iron requirement to meet fetal and placental needs. Insuffi- cient iron stores before pregnancy and inadequate iron intake during pregnancy can lead to iron deficiency ane- mia.3 The iron deficiency can be examined by assessing the serum ferritin that directly correlates with the total amount of iron stored in the body. Iron deficiency anemia is usually found when the serum ferritin is less than 12 µg/dL.4,5

A study by Milman et al (1991) suggested that neonates born to mothers receiving iron supplementation during pregnancy had higher serum ferritin than neonates born to mothers receiving placebo.6 Consistent with the previous study, Gaspar et al (1993) and Perez et al (2005) reported that neonates born to mothers with low serum ferritin tend to have low serum ferritin as well.7,8 Shao et al (2012) also found that there was a *positive* correlation between maternal serum ferritin and iron reserve in term neonates.9

Iron deficiency is associated with decreased intellectual or cognitive function. The brain region that serves as a center of memory, learning, and behavior is the hippo- campus. Iron is essential for the hippocampus to under- take dendritogenesis, synaptogenesis, neurogenesis, and synthesis of neurotrophic factors that facilitate brain growth and development commencing in intrauterine life. The main neurotrophic factor in the hippocampus for learning, memory, and behavior is brain-derived neu- rotrophic factor (BDNF). BDNF is a family of neurotro- phins that plays an important role in neuronal cell plas- ticity, oligodendrocyte growth modulation, myelin for- mation, dendritogenesis, augmentation of glutamatergic synapse transmission, and mitigation of the gamma- amino butyric acid (GABA) inhibitory signal.10,11

Animal studies have found that iron deficiency in early life leads to epigenetic changes which alter the structure of chromatin and gene expression of BDNF, thus causing a decrease in neuronal differentiation in the hippocampus. This is *also* associated with abnormalities of behavior and cognitive function.12 Acute iron deficiency that occurs in late pregnancy, delivery, and postpartum periods may result in reduction of BDNF and mitochondrial dysfunc- tion. This can lead to impairment of neuron structure, neuron electrophysiology, and behavioral development. If the iron deficiency still persists into adulthood, it may also contribute to learning and memory problems.5,13

The susceptibility of pregnant women to iron deficien- cy anemia, the positive correlation between maternal and neonatal ferritin, and the negative effect of iron deficien- cy on brain function have encouraged us to study the dif- ferences in BDNF among term newborns from mothers with normal and low ferritin.

METHODS

This was an observational study with a cross-sectional design. It was conducted from August 2015 to February 2016 and approved by the Medical Research Ethics Committee of the Dr. M. Djamil Hospital (Project Num- ber PE.07.2016). Term pregnant patients with normal leukocyte counts and willing to participate were include. The exclusion criteria were any evidence of infectious disease, vascular disease, kidney disease, diabetes melli- tus, or vaginal bleeding. Eligible patients were identified through the cooperation of three participating medical institutions located in Padang, West Sumatra, Indonesia, namely, Yarsi hospital, BMC hospital, and Hardi clinic. Those who agreed to participate in the study gave written informed consent and had their blood checked for serum

ferritin. A total of 40 pregnant women participated, 20 with normal serum ferritin (>12 ng/mL) and 20 with low serum ferritin (<12 ng/mL).

All participants were followed until delivery and the cord-blood of their newborns was examined immediately t after birth for BDNF using an enzyme-linked immuno- sorbent assay (ELISA) method *available* in the Biomedi- cal Laboratory of the Medical faculty of Andalas Univer- sity. Independent sample t-tests were used to assess sig- nificant difference (SPSS program (22.0 version).

RESULTS

Maternal characteristics are shown in Table 1.There were no differences in age, parity, Body Mass Index (BMI) or leukocyte counts between normal and low ferritin groups ($p>0.05$). However, there was a significant difference in hemoglobin between the two groups ($p<0.05$).

Neonatal characteristics are shown in Table 2. The dif- ferences in birth weight, body length, and head circum- ference among newborns from mothers with normal and low ferritin were not statistically significant ($p>0.05$). There was a significant difference in neonatal BDNF be- tween the normal and low maternal ferritin groups ($p<0.05$) (Figure 1).

DISCUSSION

As shown in Table 1, we found that those with low ferri- tin had a lower hemoglobin than those with normal ferri- tin ($p<0.05$). This is consistent with a study by Milman et al (1991) which was a placebo controlled study of 207 healthy Danish women after 16 week gestation. They reported that the placebo-treated group had a lower he-

Table 1. The maternal characteristics by maternal ferritin

Characteristics	Normal ferritin (≥12 ng/mL) N=20	Low ferritin (<12 ng/mL) N=20	p value*
Age (years)†	31.1±5.42	29.9±5.29	0.268
Parity‡			
Primipara	10 (50%)	13 (65%)	0.344
Multipara	10 (50%)	7 (35%)	
BMI (kg/m2)†	23.0±2.13	23.1±4.34	0.355
Hb (gr/L)†	109±6.9	101±12.3	0.025
Leukocytes (109/L)†	9.7±1.7	10.3±2.0	0.319

BMI: body mass index; Hb: haemoglobin.
†Data are reported as mean±SD.
‡Data are reported as percentage.
*p values are obtained by independent t-test.

Table 2. The neonatal characteristics by maternal ferritin

Sex†	Normal ferritin (≥12 ng/mL) N=20	Low ferritin (<12 ng/mL) N=20	Mean differenece * (95% CI)	p value
Men	9 (45%)	15 (75%)		
Women	11 (55%)	5 (25%)		
Birth weight (kg)‡	3.16±0.31	3.29±0.34	124 (87.1-336)	0.241
Body length (cm)‡	48.8±2.09	48.4±1.73	0.40 (0.33-1.63)	0.319
Head circumference (cm)‡	34.4±0.59	34.3±0.470	0.1 (0.04-0.44)	0.667
BDNF serum (ng/mL)‡	3.81±1.37	2.78±1.19	1.03 (0.21-1.86)	0.015

BDNF: brain-derived neurotrophic factor; CI: confidence interval.
†Data are reported as percentage.
‡Data are reported as mean±SD.
*p values are obtained by independent t-test.

Figure 1. Differences in mean level of Brain Derived Neurotrphic Factor (BDNF) between the newborns babies

from mother with normal
ferritin levels and low ferritin levels.
moglobin than the iron-treated group (p<0.001). Sao et al (2012) conducted a study in Southeastern China of 3702 pregnant women after 37 week gestation. They found that maternal anemia (Hb <11 g/dL) was present in 27.5% women and associated with low maternal serum ferritin (<20 ng/mL) in 86.9% women.6,9
Iron plays an important role in the synthesis of neuro- transmitters such as serotonin, norepinephrine, and dopamine that are essential for the synthesis of BDNF.11
Acute iron deficiency that occurs in late pregnancy and postpartum will interfere with neuronal structure and electrophysiology. As indicated above, if the iron defi- ciency persists into adulthood, learning and memory disorders may supervene.5,12 The associated decrease in BDNF expression compromises neuron differentiation in several brain areas. This underscores the importance of iron homeostasis from the commencement of central nervous system (CNS) intrauterine development.12
The observed association of lower neonatal BDNF with a low maternal ferritin is biologically plausible. Serum ferritin has a direct correlation with the total amount of iron stored in the body. Maternal iron deficiency during pregnancy has a negative effect on fetal iron homeostasis. Iron transfer from mother to fetus is a regulated process involving iron status in the maternal circulation, its transport across the placenta and subsequent transfer into the fetal circulation. The resulting iron deficiency in neo- nates may decrease the expression and function BDNF in specific areas of the brain and be reflected in a low plas- ma BDNF. Similar to the present findings, Gaspar found that cord-blood ferritin in neonates born to mothers with low serum ferritin was lower than in those born to moth- ers with normal serum ferritin.7 This is also in accordance with studies conducted by Perez in 2005 and Shao in
2012 who found that positive correlations between mater- nal serum ferritin and the iron reserve of term neonates.8,9
Given the role of BDNF in learning and memory, it is imperative that all pregnant women should be given iron replete not only to prevent any iron deficiency-related morbidity, but also to provide the mother the optimal opportunity of having intelligent offspring. The World
Health Organization (WHO) recommends a daily sup- plement of 30 to 60 mg of elemental iron as part of the antenatal care in settings where anemia in pregnant wom- en is a public health problem. A higher dose may be nec- essary if the pregnant women is diagnosed with anemia and until her hemoglobin concentration becomes nor- mal.14
A limitation of our study is the absence of neonatal fer- ritin data so that the direct relationship between maternal and neonatal ferritin could not be determined. Whether the decreased neonatal BDNF was attributable to decreased neonatal ferritin could not be determined.

Research Article

Differences in Mean Levels of Maternal Resistin Serum between Early Onset Preeclampsia (EOPE) and Late Onset Preeclampsia (LOPE)

Research Article
Differences in Mean Levels of Maternal Resistin Serum between Early Onset Preeclampsia (EOPE) and Late Onset Preeclampsia (LOPE)

INTRODUCTION

Preeclampsia is one of the major causes of morbidity and mortality of the mother and fetus. World Health Organization (WHO) reported the number of deaths caused by preeclampsia by 16% in developing countries [1]. Preeclampsia resulting 3-25 fold increased risk obstetric complications and is the cause of 30-40% of perinatal deaths in Indonesia [1].

The incidence of preeclampsia ranges between 5-10% of all pregnancies [1]. Incidence of preeclampsia in the United States, Canada and Western Europe ranges between 2-5% of all pregnancies and higher, 4-18% in some developing countries in Africa [1]. The incidence of preeclampsia in Indonesia ranged between 3-10% [3].

In Dr.M. Djamil General Hospital in Padang during the [2] year 2011, the incidence of preeclampsia was 8.31%, on 2012 was 11.47% and on 2013 was 12.02%.

Preeclampsia is divided into early onset preeclampsia (EOPE) (<34 weeks) and late onset preeclampsia (LOPE) (>34 weeks) of pregnancy based on the onset of clinical manifestations of preeclampsia. The EOPE and LOPE has a different pathogenesis. The PEAD is often associated with impaired uteroplacental perfusion caused by disruption of trophoblast invasion, while LOPE is *often* associated with the presence of extrinsic and maternal factors [4].

Preeclampsia is a protean syndrome, in which multiple [4,5] organ systems can be affected compared to the others. Preeclampsia is mainly characterized by hypertension and proteinuria or may be associated with abnormalities in laboratory test results that renal function, hepatic or hemostasis after 20 weeks of pregnancy [1].

Analysis of risk factor for preeclampsia is needed to [1,6] mitigate the adverse effects of preeclampsia. One among of these risk factor is resistin which is associated with inflammation and insulin resistance, so that resistin was a

maternal risk factor that associated with LOPE
.
Resistin is an adipose tissue-specific secretory factor
7
(ADSF), a hormone secreted by adipose tissue that induces insulin resistance in muscle and liver. Resistin stimulated by inflammatory conditions that produce proinflammatory cytokines
.
Preeclampsia is associated with inflammation and insulin
7,8
resistance which is affected by resistin. Resistin is associated with late onset preeclampsia because it was a maternal factors, so there was an increasing of maternal resistin serum levels in late onset preeclampsia
.
Previous study reported an elevated of maternal resistin
7,8
serum levels associated with a systemic inflammatory response and insulin resistance which is both of them are
2
2
increased in preeclampsia compared to normal pregnancy
.
The other study showed the mean of Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and high sensitivity C-reactive protein (hs-CRP) was higher in late onset preeclampsia than early onset preeclampsia group.
From
various studies on the above it could be estimated that the inflammatory factor and insulin resistance are associated with higher levels of resistin more dominant in late onset preeclampsia group compared to early onset preeclampsia group.
The HOMA-IR could be used as an indicator of insulin resistance, but not for inflammatory states, contrary hs-CRP could be used as an indicator of inflammation, but not for insulin resistance. Resistin is expected to describe both of them, because it was associated with insulin resistance and inflammation
7
.
MATERIALS AND METHODS
This study is an analytical cross sectional study with 20 women of early onset preeclampsia (EOPE) and 20 women of late onset preeclampsia (LOPE) who met the inclusion criteria and there were no exclusion criteria. The samples were recruited in Dr.M. Djamil General Hospital, Padang from July-October, 2015.
The inclusions criteria were women who detected early onset preeclampsia or late onset preeclampsia in obstetrics emergency ward of Dr.M. Djamil General Hospital, Padang and willing to follow the research for taking blood samples.
The exclusions criteria were diabetes mellitus (random blood

sugar as >200 mg dLG
), had suffered coronary heart disease,
chronic kidney disease, chronic liver disease and obesity
(BMI before pregnancy >25 kg mG
1
).
Maternal resistin serum was examined by enzyme-linked
2
immunosorbent assay (ELISA) method in Biomedic Laboratory
in Medical Faculty of Andalas University. Statistical analysis
was conducted by using SPSS program 20th version. The data
distribution was normal with p>0.05. The differences mean
levels of maternal resistin serum was analyzed by *using*
independent t-test.

RESULTS

Characteristics of research subjects: Forty patients of
research subjects consisted 20 patients of early onset
preeclampsia (EOPE) and 20 patients of late onset
preeclampsia (LOPE). Mean levels of maternal age in EOPE
group is 34.4±5.144 years, whereas in LOPE group is
32.9±6.324 years with p = 0.416. According to maternal age
9-12

Table 1: Characteristics of research subjects between early onset preeclampsia and late onset preeclampsia

Characteristics	EOPE (n = 20)	LOPE (n = 20)	Total (%)	p
Maternal age group				
<20 years	0 (0%)	0 (0%)	0	
20-35 years	8 (40%)	10 (50%)	45	0.537
>35 years	12 (60%)	10 (50%)	55	
Maternal age (0±SD) years	34.4±5.144	31.9±6.314		0.416
Gravidity group				
Primigravid	6 (30%)	7 (35%)	32.5	0.744
Miltigravid	14 (70%)	13 (65%)	67.5	
Gravidity (0±SD)	2.4±1.314	2.65±1.461		0.573
BMI group				
Underweight	0 (0%)	0 (0%)	0	
Normoweight	14 (70%)	12 (60%)	65	0.520
Overweight	6 (30%)	8 (40%)	35	
BMI (0±SD)	22.55±1.595	22.59±1.812		0.946

Table 2: Mean levels of maternal resistin serum in early onset preeclampsia and
late onset preeclampsia

EOPE		LOPE		
Mean	SD	Mean	SD	Variable
Maternal resistin serum (ng mLG
1
)	2.526	1.603	8.891	6.219

Table 3: Mean levels difference of maternal resistin serum in early onset
preeclampsia and late onset preeclampsia

Variable	Mean difference	CI (95%)	p
Maternal resistin serum (ng mLG
1
)	6.365	3.3835-9.3475	0.000

groups, study of subjects in early onset preeclampsia
consists 8 patients (40%) in group of maternal age

20-35 years old, 12 patients (60%) in group of maternal age >35 years old, none in group of maternal age <20 years old. Research subjects of late onset preeclampsia consists of 1 0 patients (50%) in group of maternal age 20-35 years old, 10 patients (50%) in group of maternal age >35 years old, none in group of maternal age <20 years old.

Mean levels of gravidity in early onset preeclampsia (EOPE) group is 2.4±1.314, whereas in late onset preeclampsia (LOPE) group is 2.65±1.461 with p = 0.573. According to gravidity groups, study of subjects in early onset preeclampsia consists 6 patients (30%) in group of primigravida, 14 patients (70%) in group of multigravida. Research subjects of l ate onset preecl ampsi a c onsi sts of 7 patients (35%) in group of primigravida, 13 patients (65%).

Mean levels of Body Mass Index (BMI) in early onset preeclampsia (EOPE) group is 22.551±1.595, whereas in late onset preeclampsia (LOPE) group is 22.588±1.4812 kg mG with p = 0.946. According to BMI groups, study of subjects in EOPE consists 14 patients (70%) in group of normal weight, 6 patients (30%) in group of overweight, none in group BMI underweight. Research subjects of late onset preeclampsia consists 12 patients (60%) in group of normal weight, 8 patients (40%) in group of overweight, none in group BMI underweight.

2

3

There were no statistically significant differences regarding maternal age, group of maternal age, gravidity, group of gravidity, BMI and group of BMI characteristic between those two groups with p>0.05 showed in Table 1.

Mean levels of maternal resistin serum in early onset preeclampsia and late onset preeclampsia: Mean levels of maternal resistin serum was higher in late onset preeclampsia vs

2.526±1.603 ng dLG

than early onset preeclampsia (8.891±6.219 ng mLG

1

), showed in Table 2.

Differences in mean levels of maternal resistin serum between late onset preeclampsia and early onset preeclampsia: The data distribution was normal with Kolmogorov Smirnov test. Analysis was performed with independent t-test. Table 3 showed the differences in mean levels of maternal resistin serum between early onset preeclampsia and late onset preeclampsia as 6.365 ng mLG with Confidence Interval (CI) 95% 3.384-9.347. There is a high significant differences with p<0.001.

DISCUSSION

The mean levels of maternal serum resistin in early onset preeclampsia (EOPE) group was 2.526±1.603 ng mLG whereas in late onset preeclampsia (LOPE) group was 8.891±6.219 ng mLG

(Table 2), with a mean difference

6.365 ng dLG

1
1
, 95% CI 3.384-9.347. Statistical test showed $p<0.001$, that the mean levels of maternal serum resistin significantly was higher in LOPE than EOPE (Table 3). Resistin is a hormone secreted from adipose tissue that induces insulin resistance in muscle and liver. Resistin stimulated by inflammatory conditions that produce proinflammatory cytokines. Resistin contribute to the inflammatory disorders such as coronary heart disease,
1
1
1
chronic kidney disease and liver cirrhosis as well as insulin resistance in diabetes mellitus through the activation of proinflammatory cytokines varied which depending on the organs affected
.
Insulin resistance and inflammation were the condition
7,8
associated to preeclampsia. This factor related to the role of resistin through the release of proinflammatory cytokines. The relationship between resistin and preeclampsia is connected by those which is a maternal factor in preeclampsia. Therefore, resistin is associated to late onset preeclampsia, so the levels of maternal resistin serum was increased in late onset preeclampsia
.
Inflammation and insulin resistance have been
7,8
investigated, that the comparasion between HOMA-IR and hs-CRP in EOPE and LOPE. This study reported the results that mean levels of HOMA-IR and hs-CRP were higher in LOPE compared to EOPE (HOMA IR: 4.86±5.50 vs 3.99±5.97 and hsCRP: 123.08±38.67 vs 26.54±34.7 mg LG
). This study
suggested that the inflammatory factor and insulin resistance were more dominant in LOPE compared to EOPE.
1
A s i mi l ar r es ul t s w as r epor t ed i n whi c h t he mean l evel s of maternal resistin serum was higher in preeclampsia compared to normal pregnancy related to an increase in insulin resistance and the response systemic inflammation that happened in preeclampsia particularly late onset preeclampsia
.
A cross sectional study in which two groups of pregnant
9-12
women with preeclampsia (n = 15) and normal pregnancy (n = 23) demonstrated th mean levels of maternal resistin serum was higher in preeclampsia (5.68±0.41 ng mLG
)
compared to normal pregnancy (4. 65±0. 32 ng mLG
),

p = 0.028. The mean levels of maternal resistin serum related to the mean of HOMA-IR and proinflammatory cytokines that were elevated in preeclampsia compared to normal pregnancy. T he mean of H OMA-I R (2. 5±0. 8 vs 1. 4±0. 1), IL-6 (6.34±1.02 vs 2.80±0.31) and TNF-" (1.89±0.18 vs 1.23±0.10)
.
Another cross-sectional study on two groups of
9
pregnant women, which were preeclampsia (n = 29) and normal pregnancy (n = 30), found that mean levels of maternal resistin serum was higher in preeclampsia 61.98±32.26 ng dLG
, compared to normal pregnancy 38.06±31.26 ng dLG
1
1
, p = 0.013. Insulin resistance is thought related the increasing the levels of maternal resistin serum, because the mean of HOMA-IR was higher in preeclampsia compared to normal pregnancy (4:44±4:02 vs 3.99±2.82)
.
A cross sectional study was conducted on two groups of pregnant women, which are preeclampsia (n = 50) and normal pregnancy (n = 50). The median levels of maternal resistin
1
1
10
4
serum were higher in preeclampsia 61 ng mLG
compared to
normal pregnancy 25.5 ng mLG
1
1
, p = 0.033. Insulin resistance is thought related the increasing the levels of maternal resistin serum, because the median of HOMA-IR was higher in preeclampsia compared to normal pregnancy (4.7 vs 3.6)
.
A cross sectional study was conducted on two groups of pregnant women, which are preeclampsia (n = 16) and normal pregnancy (n = 22). The mean levels of maternal resistin serum was higher in preeclampsia (12.06±0.973 ng mLG
)
compared t o n ormal p regnancy (7. 35±1. 195 ng mLG
),
p = 0.041. Inflammatory factors is thought related the increasing the levels of maternal resistin serum in preeclampsia. There was an increasing mean levels of TNF" in preeclampsia compared to normal pregnancy (15.23±0.674 vs 12.84±0.348 ng mLG
1
, p = 0.021)
. Based on

that *description,* there was a correlation between previous studies and this study
9-12
12
.
The mean levels of HOMA-IR and hs-CRP were higher in late onset preeclampsia than early onset preeclampsia, so according to those studies above, the insulin resistance and inflammation that were higher in late onset preeclampsia than early onset preeclampsia related to the levels of maternal resistin serum that was higher in late onset preeclampsia than early onset preeclampsia. It was appropriate with the results of this research that the mean levels of maternal resistin serum in late onset preeclampsia was significantly higher than early onset preeclampsia with $p<0.001$.
CONCLUSION
The mean levels of maternal resistin serum was higher significantly in late onset preeclampsia (LOPE) compared to early onset preeclampsia (EOPE).
11
1
1

Differences in brain-derived neurotrophic factor between neonates born to mothers with normal and low ferritin

INTRODUCTION

Nutrition plays a role in the intellectual development of a child during intrauterine life. The most common nutri- tional deficiency found in pregnant women is iron defi- ciency.1,2 Pregnancy is a condition that increases the iron requirement to meet fetal and placental needs. Insuffi- cient iron stores before pregnancy and inadequate iron intake during pregnancy can lead to iron deficiency ane- mia.3 The iron deficiency can be examined by assessing the serum ferritin that directly correlates with the total amount of iron stored in the body. Iron deficiency anemia is usually found *when* the serum ferritin is less than 12 µg/dL.4,5

A study by Milman et al (1991) suggested that neonates born to mothers receiving iron supplementation during pregnancy had higher serum ferritin than neonates born to mothers receiving placebo.6 Consistent with the previous study, Gaspar et al (1993) and Perez et al (2005) reported that neonates born to mothers with low serum ferritin tend to have low serum ferritin as well.7,8 Shao et al (2012) also found that there was a positive correlation between maternal serum ferritin and iron reserve in term neonates.9

Iron deficiency is associated with decreased intellectual or cognitive function. The brain region that serves as a center of memory, learning, and behavior is the hippo- campus. Iron is essential for the hippocampus to under- take dendritogenesis, synaptogenesis, neurogenesis, and synthesis of neurotrophic factors that facilitate brain growth and development commencing in intrauterine life. The main neurotrophic factor in the hippocampus for learning, memory, and behavior is brain-derived neu- rotrophic factor (BDNF). BDNF is a family of neurotro- phins that plays an important role in neuronal cell plas- ticity, oligodendrocyte growth modulation, myelin for- mation, dendritogenesis, augmentation of glutamatergic synapse transmission, and mitigation of the gamma- amino butyric acid (GABA) inhibitory signal.10,11

Animal studies have found that iron deficiency in early life leads to epigenetic changes which alter the structure of chromatin and gene expression of BDNF, thus causing a decrease in neuronal differentiation in the hippocampus. This is also associated with abnormalities of behavior and cognitive function.12 Acute iron deficiency that occurs in late pregnancy, delivery, and postpartum periods may result in reduction of BDNF and mitochondrial dysfunc- tion. This can lead to impairment of neuron structure, neuron electrophysiology, and behavioral development. If the iron deficiency still persists into adulthood, it may also contribute to learning and memory problems.5,13

The susceptibility of pregnant women to iron deficien- cy anemia, the positive correlation between maternal and neonatal ferritin, and the negative effect of iron deficien- cy on brain function have encouraged us to study the dif- ferences in BDNF among term newborns from mothers with normal and low ferritin.

METHODS

This was an observational study with *a* cross-sectional design. It was conducted from August 2015 to February 2016 and approved by the Medical Research Ethics Committee of *the* Dr. M. Djamil Hospital (Project Num- ber PE.07.2016). Term pregnant patients with normal leukocyte counts and willing to participate were include. The exclusion criteria were any evidence of infectious disease, vascular disease, kidney disease, diabetes melli- tus, or vaginal bleeding. Eligible patients were identified through the cooperation of three participating medical institutions located in Padang, West Sumatra, Indonesia, namely, Yarsi hospital, BMC hospital, and Hardi clinic. Those who agreed to participate in the study gave written informed consent and had their blood checked for serum

ferritin. A total of 40 pregnant women participated, 20 with normal serum ferritin (>12 ng/mL) and 20 with low serum ferritin (<12 ng/mL).

All participants were followed until delivery and the cord-blood of their newborns was examined immediately t after birth for BDNF using an enzyme-linked immuno- sorbent assay (ELISA) method available in the Biomedi- cal Laboratory of *the* Medical faculty of Andalas Univer- sity. Independent sample t-tests were used to assess sig- nificant difference (SPSS program (22.0 version).

RESULTS

Maternal characteristics are shown in Table 1.There were no differences in age, parity, Body Mass Index (BMI) or leukocyte counts between normal and low ferritin groups ($p>0.05$). However, there was a significant difference in hemoglobin between *the* two groups ($p<0.05$).

Neonatal characteristics are shown in Table 2. The dif- ferences in birth weight, body length, and head circum- ference among newborns from mothers with normal and low ferritin were not statistically significant ($p>0.05$). There was a significant difference in neonatal BDNF be- tween the normal and low maternal ferritin groups ($p<0.05$) (Figure 1).

DISCUSSION

As shown in Table 1, we found that those with low ferri- tin had a lower hemoglobin than those with normal ferri- tin (p<0.05). This is consistent with a study by Milman et al (1991) which was a placebo controlled study of 207 healthy Danish women after 16 week gestation. They reported that the placebo-treated group had *a* lower he-

Table 1. The maternal characteristics by maternal ferritin

Characteristics	Normal ferritin (≥12 ng/mL) N=20	Low ferritin (<12 ng/mL) N=20	p value*
Age (years)†	31.1±5.42	29.9±5.29	0.268
Parity‡			
Primipara	10 (50%)	13 (65%)	0.344
Multipara	10 (50%)	7 (35%)	
BMI (kg/m2)†	23.0±2.13	23.1±4.34	0.355
Hb (gr/L)†	109±6.9	101±12.3	0.025
Leukocytes (109/L)†	9.7±1.7	10.3±2.0	0.319

BMI: body mass index; Hb: haemoglobin.
†Data are reported as mean±SD.
‡Data are reported as percentage.
*p values are obtained by independent t-test.

Table 2. The neonatal characteristics by maternal ferritin

Sex†	Normal ferritin (≥12 ng/mL) N=20	Low ferritin (<12 ng/mL) N=20	Mean differenece * (95% CI)	p value
Men	9 (45%)	15 (75%)		
Women	11 (55%)	5 (25%)		
Birth weight (kg)‡	3.16±0.31	3.29±0.34	124 (87.1-336)	0.241
Body length (cm)‡	48.8±2.09	48.4±1.73	0.40 (0.33-1.63)	0.319
Head circumference (cm)‡	34.4±0.59	34.3±0.470	0.1 (0.04-0.44)	0.667
BDNF serum (ng/mL)‡	3.81±1.37	2.78±1.19	1.03 (0.21-1.86)	0.015

BDNF: brain-derived neurotrophic factor; CI: confidence interval.
†Data are reported as percentage.
‡Data are reported as mean±SD.
*p values are obtained by independent t-test.

Figure 1. Differences in mean level of Brain Derived Neurotrphic Factor (BDNF) between the newborns babies

from mother with normal
ferritin levels and low ferritin levels.
moglobin than the iron-treated group (p<0.001). Sao et al (2012) conducted a study in Southeastern China of 3702 pregnant women after 37 week gestation. They found that maternal anemia (Hb <11 g/dL) was present in 27.5% women and associated with low maternal serum ferritin (<20 ng/mL) in 86.9% women.6,9
Iron plays an important role in the synthesis of neuro- transmitters such as serotonin, norepinephrine, and dopamine that are essential for the synthesis of BDNF.11
Acute iron deficiency that occurs in late pregnancy and postpartum will interfere with neuronal structure and electrophysiology. As indicated above, if the iron defi- ciency persists into adulthood, learning and memory disorders may supervene.5,12 The associated decrease in BDNF expression compromises neuron differentiation in several brain areas. This underscores the importance of iron homeostasis from the commencement of central nervous system (CNS) intrauterine development.12
The observed association of lower neonatal BDNF with a low maternal ferritin is biologically plausible. Serum ferritin has a direct correlation with the total amount of iron stored in the body. Maternal iron deficiency during pregnancy has a negative effect on fetal iron homeostasis. Iron transfer from mother to fetus is a regulated process involving iron status in the maternal circulation, its transport across the placenta and subsequent transfer into the fetal circulation. The resulting iron deficiency in neo- nates may decrease the expression and function BDNF in specific areas of the brain and be reflected in a low plas- ma BDNF. Similar to the present findings, Gaspar found that cord-blood ferritin in neonates born to mothers with low serum ferritin was lower than in those born to moth- ers with normal serum ferritin.7 This is also *in* accordance with studies conducted by Perez in 2005 and Shao in
2012 who found that positive correlations between mater- nal serum ferritin and the iron reserve of term neonates.8,9
Given the role of BDNF in learning and memory, it is imperative that all pregnant women should be given iron replete not only to prevent any iron deficiency-related morbidity, but also to provide the mother the optimal opportunity of having intelligent offspring. The World
Health Organization (WHO) recommends a daily sup- plement of 30 to 60 mg of elemental iron as part of the antenatal care in settings where anemia in pregnant wom- en is a public health problem. A higher dose may be nec- essary if the pregnant women is diagnosed with anemia and until her hemoglobin concentration becomes nor- mal.14
A limitation of our study is the absence of neonatal fer- ritin data so that the direct relationship between maternal and neonatal ferritin could not be determined. Whether the decreased neonatal BDNF was attributable to decreased neonatal ferritin could not be determined.

Differences in Malondialdehyde and Catalase Activity Levels
Between Abortion and Normal Pregnancy

INTRODUCTION

Abortion is defined as a termination of a pregnancy before
20 weeks of gestation or when the fetal weight is less than
500 g. Clinically, the most frequent abortion encountered in the hospital is incomplete abortion. Patients usually come with bleeding and severe abdominal pain. The second one is threatened abortion. Threatened abortion is the most common complication in the first half of pregnancy and have incidence about 20-25%. Less than 30% of the women who experience threatened abortion will end in spontaneous abortion1.

There are many factors that associated with abortion, so it is hard to determine the exact mechanism. In spite of many possibilities, there is now a clear evidence that abortion is associated with placental oxidative stress. The abnormal placentation will lead to placental oxidative stress with resultant destructive effects on the syncytiotrophoblast. The theory has been proposed as a mechanism involved in the etiopathogenesis of abortion2.

The body has many antioxidant systems to defend the excessive Reactive Oxygen Species (ROS) production3. In a healthy body, ROS and antioxidant remain in balance. When the body has an excessive amount of ROS, Oxidative Stress (OS) then occurs. An increase in the expression of oxidative stress marker in the trophoblast was detected in abortion and this was speculated to be a cause of early pregnancy loss4.

Antioxidant system divided into enzymatic and nonenzymatic group. Catalase (CAT) is one of enzymatic antioxidant that can remove hydrogen peroxide (H2O2), prevents lipid peroxidation in the cell membrane and works as free radicals binding5. The CAT role in pregnancy is very important, in early pregnancy CAT plays a role for implantation by protecting the blastocysts from superoxide radicals in the endometrium. The CAT is also important for embryonic development and the maintenance of early pregnancy. The CAT level was found increased during pregnancy and low CAT activity in plasma or placenta found in cases of spontaneous abortion6. Another study also suggests that low antioxidant level increases the risk of spontaneous abortion7.

Free radicals are thus highly unstable molecules that have electrons available to react with a various organic substrate such as lipids, proteins and DNA8. Accumulation of lipid peroxides may cause not only tissue damage but also some biological events to accelerate the termination of pregnancy. It has been reported that ROS or lipid peroxide stimulate synthesis of PGF2α that causes uterine contraction6. Oxidative stress has been variably determined by many ways. The most reliable assessment of free radicals synthesis is using assays directly to detect the superoxide or other free radical. However, this technique is difficult and require specialized document. In another side, measurement of the products of oxidative modification product provides the most direct and possible assessment of oxidative stress. Malondialdehyde
(MDA) is a metabolite of lipid peroxides that detectable in plasma and was used as an indicator of lipid peroxidation9. This study was carried out to estimate the mean difference of CAT and MDA in normal pregnancy, threatened abortion and incomplete abortion.

MATERIALS AND METHODS

This was an observational study with cross-sectional design on February-December, 2014. The population was all pregnant women before 20 weeks gestations were enrolled in the maternity ward of Dr. M. Djamil Hospital and Dr. Reksodiwiryo Hospital, Padang, Indonesia. Subjects were taken by consecutive sampling method. Cases were those diagnosed with threatened and incomplete abortion by a specialist doctor. Meanwhile, controls were normal pregnant women without any other comorbid.

Exclusion criteria were a mother with hydatidiform mole, uterus disorder, myoma, history of provocating and recurrent abortion. After providing informed, written consent, subject's blood was taken from antecubital vein for analyzed the CAT activity and MDA concentration with spectrophotometry method in Biomedical Laboratory of Medical Faculty of Andalas University.

The significant difference among the three groups was analyzed with ANOVA test. If $p<0.05$ was found, then the analysis continued with post hoc multiple comparison test to determined the differences between two group. Statistical analysis was conducted using a computer program.

RESULTS

During the study period, 42 blood samples were collected, consisting of 14 subjects with threatened abortion, 14 subjects with incomplete abortion and 14 subjects with normal pregnancy before 20 weeks gestations. Table 1 shows the activity of CAT enzyme in incomplete abortion group was
51.10±12.20 IU mgG1, the threatened abortion group was

66.46±9.11 IU mgG1, whereas normal pregnancy group was
78.26±8.88 IU mgG1. The analysis with statistical tests ANOVA found that p<0.001. To saw the specific difference between two subjects, the analysis continued by post hoc multiple comparisons test. As presented in Table 2, there were significant differences of CAT activity between incomplete abortion group and threatened abortion (p<0.001), incomplete abortion and normal pregnancy (p<0.001), threatened abortion and normal pregnancy (p = 0.01).

Table 1: Difference of catalase activity in incomplete abortion, threatened abortion and normal pregnancy Catalase activity

Parameters	Mean±SD (IU mgG1)	95% CI	p-value*
Incomplete abortion	51.10±12.20	44.06-58.15	<0.001
Threatened abortion	66.46±9.11	61.20-71.72	
Normal pregnancy	78.26±8.88	73.13-83.38	

*p-values are obtained by ANOVA test, SD: Standard deviation, CI: Confidence interval

Table 2: Post hoc test for the difference of the mean level of catalase activity in incomplete abortion, threatened abortion and normal pregnancy

Parameters	Incomplete abortion	Threatened abortion	Normal pregnancy
Incomplete abortion	-	<0.001*	<0.001*
Threatened abortion	<0.001*	-	0.01*
Normal pregnancy	<0.001*	0.01*	-

*p-value obtained by Tukey's test to determine the difference between two groups

Table 3: Difference of malondialdehyde level in incomplete abortion, threatened abortion and normal pregnancy Malondialdehyde level

Parameters	Mean±SD (nmol mLG1)	95% CI	p-value*
Incomplete abortion	4.02±0.36	3.81-4.23	<0.001
Threatened abortion	3.46±0.39	3.24-3.68	
Normal pregnancy	2.83±0.21	2.71-2.96	

*p-values are obtained by ANOVA test, SD: Standard deviation, CI: Confidence interval

Table 4: Post hoc Test for the difference of the mean level of malondialdehyde in incomplete abortion, threatened abortion and normal pregnancy

Parameters	Incomplete abortion	Threatened abortion	Normal pregnancy
Incomplete abortion	-	<0.001*	<0.001*
Threatened abortion	<0.001*	-	<0.001*
Normal pregnancy	<0.001*	<0.001*	-

*p-value obtained by Tukey's test to determine the difference between two groups

As presented in Table 3, the mean level of MDA in incomplete abortion group was 4.02±0.36 nmol mLG1, the threatened abortion group was 3.46±0.39 nmol mLG1, whereas in normal pregnancy group was 2.83±0.21 nmol mLG1 with p = 0.00 by ANOVA test. To saw the difference between two group analysis continued with post hoc test. According to the Table 4, it was found that there were significant differences of MDA level between incomplete abortion with threatened abortion (p<0.001), incomplete abortion and normal pregnancy group (p<0.001), threatened abortion and normal pregnancy (p<0.001).

DISCUSSION

In this study, it was found that the mean of MDA level of incomplete abortion was higher than threatened abortion and normal pregnancy (p<0.05). Meanwhile, the mean level of CAT activity was lower in incomplete abortion than threatened abortion and normal pregnancy (p<0.05).

This study was accordance with a study *conducted* by Abdul-Barry et al.8 that found the serum MDA level was significantly higher in patients with a history of recurrent spontaneous abortion than women with a healthy pregnancy. Ozkaya et al.10 also reported that the spontaneous abortion before 8 weeks gestation was associated with higher serum of MDA than healthy pregnancy (66.4±13.7 vs. 40.3±16.1, p<0.01). Because MDA is a side product of lipid peroxidation, the increase in MDA level may reflect an overproduction of lipid peroxides and alter antioxidant defend mechanism.

Jauniaux et al.11 suggested the theory of how the free radical is higher in abortion than in normal pregnancy. They found that the intervillous O2 flow appeared much earlier in the abortion group so it can lead to excessive free radicals production that can cause abortion11.

The oxidative stress during pregnancy occurs in response to the fetoplacental energy demand. How the oxidative stress cause an abortion have been studied by other study. Many mechanisms have been proposed as the theory. Zhu et al.12, suggested three factors that has an impact on the pathogenesis of abortion. First, the ROS can cause

the lipid peroxidation injury in the embryo. Second, the increasing free radicals can change oxygen partial pressure in embryonic cells. And the last, increased of free radicals can lead to ischemia-reperfusion injury in the fetus12.

Actually, increased of antioxidants activity also found in early healthy pregnancy. These indicate that the oxidative stress actually also occurs in normal pregnancy but the sufficient antioxidants are defended the free radicals and prevented the damage6,13,14. In this study, the mean activity of CAT enzyme of incomplete abortion was lower than normal pregnancy and threatened abortion ($p<0.05$). It means that there were differences between the CAT enzyme activity in incomplete abortion, threatened abortion and normal pregnancy. It was suggested that the excess of free radicals and the decreased of antioxidants in this study were associated with the abortion.

Sugino et al.6 found that CAT total activity was decrease
and prostaglandin F2α synthesis was increase in decidua in cases of spontaneous abortion with vaginal bleeding. They proposed that the termination of pregnancy was caused by decreasing activity of CAT which stimulates the synthesis of prostaglandins6. This study was accordance with the study by Yigenoglu et al.15 that found the total antioxidant capacity is lower in pregnant women with history of spontaneous abortion than healthy pregnant women. It also accordance with a study conducted by Biri et al.16 that found the CAT activity was higher in abortion group than in control group (87.62±8.19 vs
44.47±3.79 IU mgG1).

CONCLUSION

Abortion group had lower antioxidant concentration and higher free radicals than normal pregnancy. There was an imbalance of free radical and antioxidant concentration in abortion.

SIGNIFICANT STATEMENT

The imbalance of free radical and antioxidant has been proposed to one of many theories that can cause abortion. This study tried to determine the comparison of antioxidant and free radical level by analyzed the catalase activity and malondialdehyde concentration in normal pregnancy and
abortion. Malondialdehyde (MDA) is a metabolite of lipid peroxides that used as an indicator of lipid peroxidation as a free radical. Meanwhile, catalase is one of antioxidant that works as free radicals binding by preventing lipid peroxidation in the cell membrane. The result of this study will determine the imbalance of free radicals and antioxidants in abortion and will help the medical field to develop the prevention of abortion.