Research Article

Placental Growth Factor Levels in Preeclampsia Compared to Normal Pregnancy

Kadar Placental Growth Factor pada Preeklampsia dan Kehamilan Normal

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Abstract

Objective: To identify and analyze the differences in the levels of PlGF in preeclampsia and normal pregnancy.

Method: This was a cross-sectional observational qualitative study of PlGF in preeclampsia and normal pregnancy. The number of samples in case and control group was 30 in each groups.

Result: We found that mean of maternal age in the preeclampsia group was 28.53 years and 25.23 years in the control group. Mean parity in preeclampsia and control group was 2.33 and 1.56, respectively. Mean hemoglobin level in preeclampsia and control group was 11.97 and 11.99, respectively. Mean maternal blood glucose level was 87.0 in the preeclampsia group, and 87.9 in the control group. In the preeclampsia group mean urea concentration was 16.45, while it was 22.78 in the control group. Mean creatinine level was 0.92 in the preeclampsia group and 0.64 in the control group. Mean SGPT and SGOT in the preeclampsia group was 23.36 and 21.97, while in the control group was 29.86 and 26.20. Test results showed that PlGF levels was significantly different between the preeclampsia and control group. Mean PlGF in the preeclampsia and control group was 42.10 and 452.33 respectively, with p <0.001.

Conclusion: We can conclude that the level of PlGF in the preeclampsia group is lower than the normal pregnancy group.

[Indones J Obstet Gynecol 2015; 2: 76-80]

Keywords: angiogenic factors, PlGF, preeclampsia

Abstrak

***Tujuan**: Mengetahui dan menganalisis perbedaan kadar PlGF pada preeklampsia dan kehamilan normal.*

***Metode**: Penelitian ini adalah penelitian kuantitatif observasional menggunakan pendekatan potong lintang kadar PlGF pada preeklampsia dan kehamilan normal. Jumlah sampel pada masing-masing kelompok kasus dan kontrol adalah 30 orang.*

***Hasil**: Dari hasil penelitian kami, didapatkan bahwa rerata variabel umur ibu hamil pada kelompok preeklampsia adalah 28,53 tahun, sedangkan pada kelompok kontrol adalah 25,23 tahun. Rerata paritas untuk kelompok preeklampsia dan kontrol adalah 2,33 dan 1,56. Rerata kadar hemoglobin adalah 11,97 untuk kelompok preeklampsia dan 11,99 untuk kelompok kontrol. Untuk rerata GDS ibu pada kelompok preeklampsia 87,0, kelompok kontrol adalah 87,9. Pada kelompok preeklampsia didapatkan rerata ureum sebesar 16,45 dan 22,78 untuk kontrol. Rerata kreatinin 0,92 untuk preeklampsia dan 0,64 untuk kelompok kontrol. Pada rerata SGPT dan SGOT pada kelompok preeklampsia 23,36 dan 21,97, sedangkan pada kelompok kontrol sebesar 29,86 dan 26,20. Hasil pemeriksaan kadar PIGF menunjukkan bahwa terdapat perbedaan signifikan antara kelompok preeklampsia dan kelompok kontrol. Pada kelompok preeklampsia didapatkan rerata PlGF sebesar 42,10 dan 452.33 untuk kontrol dengan p<0,001.*

***Kesimpulan**: Berdasarkan analisis hasil penelitian didapatkan kesimpulan bahwa kadar PlGF pada kelompok preeklampsia lebih rendah daripada kelompok dengan kehamilan normal.*

[Maj Obstet Ginekol Indones 2015; 2: 76-80]

***Kata kunci**: faktor angiogenik, PlGF, preeklampsia*

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INTRODUCTION

Preeclampsia is a contributor to maternal as well as perinatal morbidity and mortality, with rate of preeclampsia in all pregnancy estimated to be 5%.[1,2] Although the etiology of preeclampsia is definitely unclear, it is suspected that the presence of endothelial dysfunction plays an important role in the development of preeclampsia.[2-4] Preeclampsia is a clinical diagnosis, with the classic definition of preeclampsia consisting of three components; hypertension (blood pressure >140/90 mmHg in women who previously had normal blood pressure); proteinuria (>300mg/24 hours or +2 on urinalysis examination in the absence of urinary tract infection); and edema. However, a recent consensus excluded edema as a criterion for diagnosing preeclampsia. So far, screening tests and prevention of preeclampsia have not been available. Complications resulting from preeclampsia include eclampsia, hemolysis, elevated liver enzymes, low platelets (HELLP syndrome), disseminated intra-

vascular coagulophaty (DIC), hypertensive emergency, hypertensive encephalopathy and blindness in cortical regions.[4,5]

In the United States, preeclampsia 23.6 cases occurs in every 1000 delivery.[6,7] The incidence of preeclampsia in Indonesia was 3-10% and accounted for 39.5% of maternal mortality in 2001 and increased to 55.56% in 2002.[8]

Although preeclampsia has been studied extensively for numerous decades, the etiology and pathogenesis of this disease remains unclear. Currently many theories trying to explain the etiology and pathogenesis of preeclampsia exist, including genetic predisposition, thrombophilia, endocrinopathy, vasculopathy, placental ischemia, oxidative stress, and immune maladaptation.[9,10]

Preeclampsia is mostly believed to occur in two stages. The first stage is the preclinical stage, which is a process of endothelial disruption and inadequate cytotrophoblast invasion of the spiral arteries in the myometrium. This poor placentation process leads to ischemia and hypoxia in the placenta. The second stage occurs in late pregnancy, where the presence of placental oxidative stress causes the release of anti-angiogenic proteins such as soluble Fms-like Tyrosine Kinase-1 (sFlt-1), prostaglandins and cytokines into the maternal circulation. On the other hand, the state of oxidative stress will suppress the production of pro-angiogenic factors, including placental growth factor (PlGF) and vascular endothelial growth factor (VEGF).[11]

There was growing evidence about the imbalance between angiogenic factors, such as PlGF and VEGF, with angiogenesis inhibiting factors, such as sFlt-1 and soluble Endoglin (zinc), in the pathogenesis of preeclampsia. sFlt-1 was found to be responsible for the syndrome of preeclampsia. In a study of animals given exogenous sFlt-1, symptoms of preeclampsia such as hypertension, proteinuria and kidney damage was shown. sFlt-1 is increased in preeclampsia, and decreased to normal levels after birth. In contrast with sFlt-1, PlGF concentrations decreases in preeclampsia.[12] Levine et al reported that the increase of sFlt-1 as a result of endothelial dysfunction caused a decrease of free PlGF in serum preeclampsia.[13] Soluble Fms-like Tyrosine Kinase-1 is an antagonist of PlGF and VEGF, are bound and prevents the interaction of PlGF and VEGF in endothelial surface receptors.[14,15]

The balance between PlGF, VEGF (pro-angiogenic factors) and sFlt-1 (anti-angiogenic factors) is important in the process of angiogenesis, vasculogenesis and placental development during pregnancy.[16]

PlGF is a member of the VEGF polypeptide family. In recent times, it has been observed that serum PlGF level at mid-pregnancy is significantly lower in obese women. It has been established that PlGF could be an effective predictor of preeclampsia in the early second trimester of pregnancy. However, some researchers also considered PlGF to be more effective as a biomarker in high-risk populations although the predictive value of these biomarkers appear to be limited in early onset preeclampsia.[17,18] Based on this, the researchers intend to conduct a study to determine and analyze the differences in the levels of PlGF in preeclampsia and normal pregnancy.

METHODS

We carried out a cross-sectional observational qualitative study of PlGF in preeclampsia and normal pregnancy. This study was conducted in the Department of Obstetrics and Gynecology, RSUP Prof. Dr. RD Kandou from September to October 2013.

Patients with preeclampsia and normal pregnancy presenting in the outpatient unit who meet the inclusion criteria and provided informed consent after receiving explanation about the procedure of this study. Inclusion criteria include women with preeclampsia or normal pregnancy, at term, gave birth in RSUP Prof. Dr. RD Kandou, Manado, and willing to participate in this study. Women having chronic diseases such as diabetes mellitus, cardiovascular or kidney abnormality, chronic hypertension, multiple pregnancy, IUFD, premature rupture of fetal membranes, intrauterine infection, congenital malformation, or placental abnormalities (hemangiomatous placenta or placenta previa) were excluded from this study. The sample size needed to meet 90% power of this study was 30 subjects in each preeclampsia and control group.

About 10 ml of blood sample was taken from the cubital vein, 5 ml for PlGF serum assay and 5 ml for blood sugar, SGOT, SGPT, ureum, creatinine, hemoglobin, leukocyte and total protein test. The blood tests, except for PlGF assay, were done in RSUP Prof. Dr. RD Kandou laboratory. Blood for PlGF assay was centrifuged at 2000-3000 rpm for

15 minutes and then stored in the freezer with a temperature of -20°C before it was sent to Prodia Laboratory in Jakarta in an ice pack to maintain the temperature. PlGF was tested quanitatively using ELISA.

Serum PlGF data from the preeclampsia and control group was collected and compared statistically using t-test. Data analysis was done using SPSS 17.0 for Windows.

RESULTS

The subjects of this study were 60 pregnant women at term that met the inclusion criteria. They were allocated into 2 groups, preeclampsia as the case group and normal pregnancy as the control group. Baseline characteristics of our subjects are shown in Table 1.

From this study, we found that the mean maternal age in the preeclampsia group was 28.53 years, and 25.23 years in the control group. The mean parity in preeclampsia and control group was 2.33 and 1.56, respectively. Mean hemoglobin and maternal blood glucose was similar in both groups. Moreover, in the preeclampsia group mean urea level was 16.45, while it was 22.78 in the control group. Mean creatinine was 0.92 in the preeclampsia group and 0.64 in the control group. Mean SGPT and SGOT in the case group was 23.36 and 21.97, while it was 29.86 and 26.20 in the control group.

Table 1. Baseline Characteristics

Characteristics	Control Group		Preeclampsia	
	Total (n)	Percentage (%)	Total (n)	Percentage (%)
Age				
<20 years	14	56.67	14	56.67
20-24 years	3	10.0	3	10.0
25-29 years	5	16.67	5	16.67
30-35 years	8	26.67	8	26.67
Weight				
<60 kg	7	23.3	7	23.3
60-79 kg	20	66.6	20	66.6
80-100 kg	3	10.0	3	10.0
>100 kg	-	-	-	-
Education level				
High school	2	6.67	2	6.67
Junior high school	5	16.67	5	16.67
Senior high school	19	63.33	19	63.33
College	4	13.33	4	13.33
Occupation				
Housewive	14	56.67	14	56.67
Civil servant	6	20.0	6	20.0
Private company	10	33.33	10	33.33
Ethnic group				
Minahasa	20	66.6	20	66.6
Sangihe	4	13.33	4	13.33
Gorontalo	4	13.33	4	13.33
Javanese	2	6.67	2	6.67

Table 2. Distribution of Study Variables

Variables	Group	N	Mean
Age (yo)	Preeclampsia	30	28.5
	Control	30	25.2
Parity	Preeclampsia	30	2
	Control	30	2
Hb (gr/dl)	Preeclampsia	30	11.9
	Control	30	11.9
Blood glucose (mg/ dl)	Preeclampsia	30	89
	Control	30	87
Ureum (mg/ dl)	Preeclampsia	30	16.4
	Control	30	22.7
Creatinin (mg/dl)	Preeclampsia	30	0.9
	Control	30	0.6
SGOT (U/l)	Preeclampsia	30	23.3
	Control	30	21.9
SGPT (U/l)	Preeclampsia	30	29.8
	Control	30	26.2

Serum PIGF level from preeclampsia and control group was collected and compared statistically using SPSS 17.0 for Windows. Mean PlGF in preeclampsia and control group was 42.10 and 452.33, respectively. Statistical analysis showed that PlGF levels in the preeclampsia and control group was significantly different. Using Mann-Whitney test we found that the difference of PIGF level between the control and preeclampsia group was statistically significant ($p<0.01$).

DISCUSSION

Preeclampsia is a complication of pregnancy presenting in the form of a syndrome with serious consequences for the mother and fetus. Incidence of preeclampsia was found to be 7% to 10%. This syndrome is characterized by the presence hypertension, proteinuria and multi-organ failure. Preeclampsia is considered as a multi-system syndrome, characterized by vasoconstriction, metabolic changes, endothelial dysfunction, activation of the coagulation cascade, and increased inflammatory response.[1]

Although preeclampsia has been studied extensively for several decades, the etiology and pathogenesis of this disease is still unclear. Angiogenesis is one of the important processes that play a role in the development of the vascular system of the placenta. Among several angiogenic factors, VEGF and PlGF play an important role in the vascular system of the placenta. It has been proven that an imbalance between angiogenesis factors such as VEGF and PlGF and antagonist of angiogenesis factors, such as sFlt-1, plays a major role in the pathogenesis of preeclampsia. Angiogenic factors PlGF and VEGF have an essential function for placental development and the effectiveness of endothelial cells.[9,10]

In normal pregnancy, serum PlGF concentration increases at 8-12 weeks gestation, peaks at 29-32 weeks gestation, and then declines at 33-40 weeks gestation.[11] In several studies, PlGF showed low levels in preeclampsia. PlGF levels in women who develop preeclampsia was found to be significantly lower than in normotensive pregnancies at 13-16 weeks gestation until the beginning of labor. In addition it was also reported that at the beginning of the second trimester (10 or 11 weeks), levels of PlGF in pregnancies that would evolve into preeclampsia was lower compared to normal pregnancy.[11,16]

PlGF has been proposed as a marker and mediator of endothelial dysfunction in preeclampsia. Some studies found that the concentration of PlGF in preeclampsia is 3 to 10 times lower than in normal pregnancy. PlGF concentration in preeclampsia begins to fall at 9-11 weeks before the onset of hypertension and proteinuria; or at least 5 weeks before the onset of this condition.[16]

Many studies have evaluated the levels of PlGF in preeclampsia compared with normotensive pregnancies. Krauss et al reported that out of all pregnancies with preeclampsia, 27.3% had PlGF levels below 200 pg/ml.[19] Bersinger et al found levels of PlGF in preeclampsia to be significantly lower than in normotensive pregnancy at term.[20] Likewise, Shen et al found levels of PlGF in pregnancies with preeclampsia to be significantly lower compared to normotensive pregnancies at the same gestational age.

This study is an observational study using quantitative cross-sectional approach to prove the existence of differences in the angiogenic factor (PlGF) in normal pregnancies and pregnancies with preeclampsia.

We found that the difference between mean levels of PlGF between the preeclampsia group and

normotensive group was significant ($p<0.05$), where levels of PlGF in the preeclampsia group was 42.10 pg/dl, and in the normotensive group was 452.33 pg/dl.

There are several explanations regarding the decreased levels of PlGF in pregnancies with preeclampsia. First, low levels of PlGF occur due to inadequate trophoblast proliferation into the maternal spiral arteries in early pregnancy. It may occur due to impaired renal function, capillary thickening, decreased perfusion and other mechanism. Looking at the results of this study and some previous studies, we can conclude that PlGF is very potential to be developed as a predictor of preeclampsia. By evaluating the PlGF levels in pregnant women as early as possible, we can predict the occurrence of preeclampsia and possibly prevent it.

Although the results of this study showed a significant difference between preeclampsia with normotensive pregnancies, this research has some limitations including the limited number of samples, so we could not determine the cut-off point of the occurrence of preeclampsia. Moreover, the study was limited to proangiogenic factor levels (PlGF) in term pregnancy. Research on early gestation until term is needed to allow us to clearly see the difference in the levels of angiogenic factors in normal pregnancies and pregnancies with preeclampsia. Furthermore, PlGF concentration is influenced by many factors, such as the presence of ischemic tissue, malignancy, inflammation and multiple other abnormalities. Therefore, changes in PlGF concentration are not only specific to preeclampsia.

CONCLUSION

Based on our study results, we can conclude that the level of PlGF in pregnancy complicated by preeclampsia is lower than in normal pregnancy. A multicenter study involving more participants who were observed from the beginning of pregnancy until delivery is needed in order to apply the results on the population of pregnant women in preventing preeclampsia.

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