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RESEARCH ARTICLE

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Differentiating Maternal Angiogenesis Factors between Early and Late Onset Preeclampsia: Higher sflt-1 in Early Onset Preeclampsia, Lower PlGF and Higher sflt-1/PlGF Ratio in Late Onset Preeclampsia

Joserizal Serudji[1], Vaulinne Basyir[1],Tara Fadhillah[2]

[1]*Fetomaternal Division, Obstetrics and Gynecology Department, Faculty of Medicine, Universitas Andalas, Padang, Indonesia*
[2]*Obstetrics and Gynecology Department, Faculty of Medicine, Universitas Andalas, Padang, Indonesia*

Background: Early onset preeclampsia (PE) is considered a fetal disorder that is associated with placental dysfunction. While late onset preeclampsia is considered a maternal disorder that associated with a normal placenta. An imbalance of angiogenesis factors, namely soluble Fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) is not only a strong predictor of PE but can also be a predictor of poor outcomes or adverse complications of PE. This study was conducted to analyze the difference between angiogenesis factor sFlt-1 and PlGF in maternal serum between patients with early and late onset PE.

Material and Methods: This was a cross-sectional study involving pregnant women with PE who were ≥18 years old and gestational age >20 weeks had singleton pregnancies. Subjects who had a major morphological abnormality of a fetus diagnosed with USG and chromosomal abnormality of the fetus were be excluded. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were measured using a digital blood pressure measuring instrument. sFlt-1 and PlGF levels were measured using enzyme linked immunosorbent assay (ELISA).

Result: This study showed the level of maternal serum sFlt-1 was 7522.95 pg/mL and the level of maternal serum PlGF was 222.25 pg/mL. There was a difference between sflt-1, PlGF, and the sflt-1/PlGF ratio (p=0.00; p=0.00; p=0.00) in early and late onset of PE where at early onset PE was found higher sflt-1 and late-onset PE had lower PlGF and higher sflt-1/PlGF ratio.

Conclusion: There are differences in sFlt-1, PlGF, and sFlt-1/PlGF in early and late onset PE, as higher sflt-1 is found in early onset preeclampsia, while lower PlGF and higher sflt-1/PlGF ratio are found in late onset preeclampsia.

Keywords: *early onset, late onset, preeclampsia, maternal angiogenesis factor, Flt-1, PlGF*

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Corresponding Author:
Joserizal Serudji
Fetomaternal Division
Obstetrics and Gynecology Departement
Faculty of Medicine, Universitas Andalas
Limau Manis, Pauh, Padang 25166, Indonesia
e-mail: jrserudji@yahoo.co.id

Cell and Biopharmaceutical Institute

Introduction

Preeclampsia (PE) is a unique multisystem disorder in pregnancy characterized by vascular endothelial dysfunction which threatens mother and fetus condition in 2-5% of all pregnancies. PE causes about 50,000 maternal deaths and 500,000 perinatal deaths per year.[1–3] Clinical management of women at risk of PE requires improvement to minimize the significant risk of adverse maternal and perinatal outcomes associated with preeclampsia.[4]

Screening for PE is carried out by measuring blood pressure and urine protein. There is no standard practice for laboratory examinations in early pregnancy that can predict the risk of preeclampsia. Meanwhile, studies show that there are prevention options in high-risk groups, so sensitive laboratory tests are needed to screen for PE before it manifests.[5] Currently, it has been suggested that angiogenic/antiangiogenic factors can be used as biomarkers to identify patients at risk of developing preeclampsia.[6]

The pathomechanism of PE starts from abnormal placental development from early pregnancy, which is associated with impaired decidualization, vasculogenesis, angiogenesis, and spiral artery remodeling which causes endothelial dysfunction due to abnormal cell death. The process of abnormal cell death in the placenta causes insufficiency of implantation which then causes placental hypoxia which induces increased production of soluble FMS-like tyrosine kinase (sFlt)-1, an antiangiogenesis factor that bind vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) thereby reducing the levels of angiogenesis factors namely free VEGF and PlGF in the blood or known as the occurrence of angiogenesis clashes.[7,8]

In the last 2 years, sFlt-1/PlGF has been established in routine obstetrical practices in Germany and Europe to predict and diagnose PE in high-risk women.[9] Women with an Sflt-1/PlGF ratio of 85.52 would develop poor outcomes or complications of PE. This illustrates the importance of sFlt-1/PlGF as a relative measure of pre-existing PE and PE with complications.[10]

Early onset PEis considered a fetal disorder that is typically associated with placental dysfunction. While late onset PE is considered a maternal that is more often associated with a normal placenta. Angiogenetic factors had been assessed in early onset PE due to their role in pathomechanism. Meanwhile, in late onset PE, where the pathomechanism is based on maternal disease, study results still vary regarding the role of sFLt-1 and PlGF. However, a relationship between maternal comorbidities such as atherosclerosis, obesity, and thyroid with an increase in the sFLt-1/PlGF ratio was also found.[11]

In Indonesia, there are still few studies that assess the level of sFlt-1, PlGF, and sFlt-1/PlGF ratio serum levels in early and late-onset PE which has different mechanisms. Therefore, this study was conducted to determine the difference between angiogenesis factor sFlt-1 and maternal serum PlGF in early and late-onset PE.

Materials and methods

Study Design and Subject Recruitment

A prospective observational study using a cross-sectional design was conducted at Dr. M. Djamil Hospital, Padang from January to December 2022. Subjects included in this study were pregnant women above 18 years old with a gestational age of 20 weeks until delivery, who were diagnosed with PE features based on clinical symptoms and laboratory examination results. Subjects who had major morphological abnormality of fetuses diagnosed with USG and chromosomal abnormality of fetuses were be excluded from this study. Forty included subjects were then divided into 2 groups, 20 subjects with early onset and 20 subjects with late onset PE. The protocol of this study was approved by The Ethical Committee of Faculty of Medicine, Universitas Andalas (No. 792/UN.16.2/KEP-FK/2022). Informed consent was signed by pregnant mothers or the research subject's family member.

Clinical Data Measurement

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) of subjects were measured during the hospital admission, both from the emergency department and the outpatient clinic. The measurements were performed using a digital blood pressure measuring instrument.

sFlt-1 and PlGF Measurement

sFlt-1 and PlGF were measured using enzyme linked immunosorbent assay (ELISA) and performed in the iomedical laboratory at Faculty of Medicine, Universitas Andalas, Padang. Approximately 5 mL of venous blood samples were taken and centrifuged for 30 minutes; then the blood serum was stored at -40^{o}C until the sFlt-1 and PIGF measurements were analyzed. sFlt-1 and PIGF were analyzed using human vascular endothelial growth factor receptor 1 (VEGFR1)/FMS-like tyrosine kinase 1 (FLT1)

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ELISA kit (Elabscience, Houston, TX, USA) and human placental growth factor (PGF) ELISA kit (Elabscience). Then, 50 µL standard were added to standard well. After that, 40 µL sample and 10 µL Human PGF antibody were added to sample wells; then 50 µL streptavidin-HRP was added to both sample wells and standard wells and mixed. The plate was incubated 60 minutes at 37°C and then washed 5 times with wash buffer. After another soak, wash, aspirate, and blotted, substrate solution was added. Then, the plate was incubated, and the stop solution was added to see blue color will change into yellow. The optical density (OD) value of each well was determined using a microplate reader set to 450 nm within 10 minutes.

Statistical Analysis

The Saphiro-wilk test was used to determine the normality of the data between sFlt-1, PIGF levels, and the ratio of sFlt-1 and PIGF in early and late-onset PE. Statistical package for the social sciences (SPSS) ver. 24 was used for data analysis. The $p<0.05$ was used to determine the study's significance level with a 95% confidence interval (CI).

Results

PE Subjects' Characteristics

In the early onset PE group, the mean of age was 32.15±5.62 years old, the majority were multiparous (60%), had a mean of gestational age was 30.65±2.15 weeks, mean of SBP was 170.90±11.24 mmHg, mean of DBP was 104.35±7.29 mmHg and mean of MAP was 125.53±7.23 mmHg. Meanwhile, the mean age of late-onset PE subjects was 28.90±6.40 years old, with the majority also multiparous (60%), had a mean of gestational age was 36.40±1.72, mean of SBP was 170.40±7.27mmHg, mean of DBP was 106.30±6.82 mmHg and mean of MAP was 127.65±5.62 mmHg (Table 1). Normality test of sFlt-1, PlGF-1 and ratio of sFlt-1/PIGF in early and late onset PE were analyzed with Shapiro-Wilk test. The data was normally distributed.

Difference of sFlt-1, PlGF, and sFlt-/PlGF Ratio in Early and Late Onset PE

The result of this study showed the difference between sFlt-1, PlGF-1, and the ratio of sFlt-1 and PIGF in early and late onset PE. The mean level of sFlt-1 in early onset PE was higher than late onset (7522.95±729.26 pg/mL *vs*. 5955.00±844.47 pg/mL). There was a difference between sFlt-1 levels in early and late onset of PE (p=0.000). It was also found that the mean PlGF-1 level in early onset PE was higher than in late onset PE and the difference was statistically significant (222.25±32.89 pg/mL *vs*. 102.25±6.89 pg/mL; p=0.000). The value of the sFlt-1/PIGF ratio was also found to be higher in the late onset group than the early onset group where the difference was also significant (34.46±5.79 *vs*. 58.13±6.41; p=0.000) (Table 2).

Table 1. Characteristic of participants with early onset and late PE.

Characteristic	Preeclampsia	
	Early Onset	Late Onset
Parity		
Multipara, n (%)	12 (60)	13 (65)
Primipara, n (%)	8 (40)	7 (35)
Age (years), mean±SD	32.15±5.62	28.90±6.40
GA (weeks), mean±SD	30.65±2.15	36.40±1.72
SBP (mmHg), mean±SD	170.90±11.24	170.40±7.27
DBP (mmHg), mean±SD	104.35±7.29	106.30±6.82
MAP (mmHg), mean±SD	125.53±7.23	127.65±5.62

Table 2. The difference between sFlt-1, PlGF-1 and ratio of sFlt-1 and PIGF in early and late onset PE.

Factor	Preeclampsia	
	Early Onset	Late Onset
sFlt-1 serum (pg/mL)	7522.95±729.26*	5955.00±844.47*
PlGF serum (pg/mL)	222.25±32.89*	102.25±6.89*
sFlt-1/PlGF	34.46±5.79*	58.13±6.41*

*p-value<0.05 in independent T-test.

Discussion

In this study, both groups of subjects had almost similar characteristics with most of them being multipara and having an equivalent mean of SBP, DBP, and MAP. Although PE generally occurs in primipara, weight gain in multipara also

increased the incidence of PE which may have contributed to this result. In this study, the early onset PE group had a mean age of above 30 years, while the late onset PE group had a mean age of under 30 years.[12] Chronic hypertension and older age are usually associated with early onset PE as found in this study.[13]

The mean level of sFlt-1 and PlGF is higher in the early onset PE group than in late onset PE. But, a higher sFLt-1/ PlGF ratio in the late onset PE group. Previous studies on early onset PE also found similar results with higher sFlt and lower PlGF. A study involving 80 pregnant women found a higher median sFlt-1 value of 8690 pg/mL in early-onset PE.[14] PlGF levels during pregnancy were around 353 pg/mL which were below normal (222.25±32.89 pg/mL).[15] Lower PlGF levels were more likely to develop early onset PE.[16] Previous studies on late onset PE found sFlt-1 levels were 6990 pg/mL and mean PlGF levels were 38.7 pg/mL.[17] There was also an increase in sFlt-1 and a decrease in PlGF in late-onset PE.[18]

The syncytiotrophoblast surface layer, which is in direct contact with the mother's blood, is very susceptible to damage where this layer is a source of 'pre-eclampsia biomarkers' such as sFlt-1. An increase in sFlt causes a decrease in free PlGF levels because sFlt binds to PlGF. This is the reason why there is an increase in sFlt-1 levels and a decrease in PlGF levels in early-onset PE.[4,19] In contrast to early onset, disturbances in angiogenic/anti-angiogenic factors occur due to lifestyle and maternal metabolic disease which induce syncytiotrophoblastic stress in late onset PE.[20,21] Therefore, in this study, an increase of sFLt-1 and sFLt-1/ PlGF ratio and a decrease of PlGF in late onset PE were also found.

In this study, there was a significant difference between sFlt-1 in early and late onset of PE. This is similar to a previous study which found higher level of sFlt in early onset than late onset PE (4.69±0.96 pg/mL *vs*. 2.39±0.57 pg/mL).[22] The sFlt-1 values were higher in early than late onset PE (9.51±0.71 pg/mL *vs*. 8.89±0.78 pg/mL).[23] A study conducted 44 pregnant women in Nepal also found that the sFlt-1 level was higher in early onset PE (3017.11 pg/mL vs. 2269.76 pg/mL; p=0.000).[6]

PlGF-1 levels in late onset PE were lower than in early onset PE. This is similar to the study which found that PlGF decreased with increasing gestational age.[24,25] Similar results were also found on 44 preeclamptic pregnant women in Nepal which found a higher PlGF value at early onset than late onset (95.24 vs. 80.12 pg/mL; p=0.05).[6]

The function of PlGF is to build up the development and maturation vascular system of the placental. In PE, the levels of PlGF are found to decrease due to the role of increased sFlt-1 which binds them. Besides that, in normotensive pregnancy, sFlt-1 levels increase and PlGF levels decrease during the last two months of gestation. These changes occur earlier and are more pronounced in women who will develop PE.[26] Therefore, in this present study, it was found that PlGF values were also getting lower at the late-onset because physiologically, this molecule was decreasing as gestational age increased.

In this study, it was found that there was a difference between sflt-1/PlGF ratio in the early and late onset of PE. Early onset PE tends to have a lower mean value than late onset PE. This is in line with a study that revealed that the sFlt-1/PlGF ratio was higher in late onset than early onset PE.[24] Late onset PE is affected by several factors, including the environment and maternal conditions. A study showed that sFLT-1 and PlGF were not only influenced by implantation but also maternal conditions such as atherosclerosis, obesity, and thyroid which resulted in a higher sFLT-1/PlGF ratio of late onset PE found in this study.[11]

This is a single-centered study, so this might not represent the general population. In addition, with fewer samples, the risk of bias could be greater because samples tend not to be heterogeneous. This study also did not include healthy pregnant women of the same gestational age so this factor could be a bias due to its physiological influence on sflt-1 and PlGF levels. Moreover, this study did not assess the relationship between angiogenetic factors and maternal comorbidities in late onset PE. Therefore, further study should include populations from multiple centers with a greater number of participants and also healthy pregnant women to compare sFLt-1 and PlGF levels regarding maternal factors.

Conclusion

There were differences between sFlt-1, PlGF, and the sFlt-1/ PlGF ratio in early and late-onset of PE. The sFlt-1 levels were higher in early onset than late onset PE. PlGFt-1 levels were lower in late-onset PE than early-onset PE. The sFlt-1/ PlGF ratio was higher in late-onset than in early onset PE.

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Authors contribution

JS performed material preparation and data collection. JS, VB and TF contributed to the study conception, design, and analysis. JS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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