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DIFFERENCES RATIO LEVEL SOLUBLE FMS-LIKE TYROSINE KINASE-1 AND PLACENTAL GROWTH FACTOR EARLY AND LATE ONSET ON PREECLAMPSIA AND NORMAL PREGNANCY

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A B S T R A C T

Preeclampsia is a major maternal morbidity and mortality worldwide including in Indonesia. PIGF concentrations were found to be lower and sFlt-1 to be higher in patients with preeclampsia than normal pregnancy. Futher, this factor has been proposed as a parameter that can help identify women with potentially preeclampsia.This study aims todeterminethe differences ratio level soluble rate fms-like tyrosine kinase-1 and placental growth factor early and late onset on preeclampsia and normal pregnancy. The cross sectional study design was conducted in RSUP M.Djamil, Rasidin Hospital, Reksodiwiryo Hospital and Biomedical Laboratory of Andalas University from July 2017 until January 2018. The sample of this study was pregnant women>20-42 weeks pregnancy totalling 80 people by consecutive sampling.Sample was divided into 3 groups. SFlt-1 and PlGF levels tested using ELISA test. Test the normality of data by Kolmogrov-Smirnov test by using unpaired T test.The results showed median sFLT-1 levels in the early onset group with normal pregnancy with p= 0,88, median sFLT-1 levels in the late onset group with normal pregnancy with p= 0,01 and median levels of sFLT-1 in the early onset group with late onset with p= 0.34. Mean of PlGF in the early onset group with normal pregnancy with p=0,30, mean of PlGF in the late onset group with normal pregnancy with p= 0.63, and mean of PlGF in the early onset group with late onset with p = 0.27. The conclusion of this study was that there was a difference ratio level Soluble Fms-Like Tyrosine Kinase-1 late onset in preeclampsia and normal pregnancy.

I. INTRODUCTION

Preeclampsia is a major source of maternal morbidity and mortality worldwide including Indonesia. Regulating failure and imbalance of proangiogenic and placental anti-angiogenic agents such as placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) have been shown to play an important role in the pathogenesis of preeclampsia. PIGF concentrations were found to be lower whereas sFlt-1 plasma concentrations were found to be higher in patients with preeclampsia compared with normal pregnancies, so this factor has been proposed as a parameter that can help identify women with potential of experiencing preeclampsia.[1]

The high sFlt-1 antiangiogenic protein in cases of preeclampsia reduces the circulation of PIGF and VEGF placental proangiogenic proteins that the body needs for blood vessel maintenance. The resulting endothelial dysfunction may subsequently lead to hypertension and proteinuria.[2]

Rahmi'sstudy, et al (2016) showed that maternal serum sFlt-1 levels between early onset and late onset preeclampsia were higher / higher compared to normal pregnancies. Another similar study of the relationship of biological factors to serum levels of sFlt-1 in pregnant women with severe preeclampsia (PEB) explained that maternal serum SFlt-1 levels of PEB were found to be higher in high-risk ages and mothers with prior history of preeclampsia.[2]

The incidence of eclampsia in developing countries is around 1 case per 100 pregnancies to 1 case per 1700 pregnancies. In African countries such as South Africa, Egypt, Tanzania, Ethiopian dam varies around 1.8% to 7.1%. In Nigeria the prevalence is around 2% to 16.7%.[3]

In Indonesia severe preeclampsia and eclampsia are the causes of maternal mortality ranging from 1.5 percent to 25 percent, while infant mortality is between 45 percent and 50 percent. Preeclampsia included in hypertension in pregnancy (HDK) occupies the second highest position after bleeding which causes maternal death. Although not the most common cause of mortality rate in Indonesia, the proportion of events has increased compared to the other two causes of mortality rate in Indonesia, namely bleeding and infection which tend to decrease from the previous year.[4]

The cause of maternal death in the city of Padang in 2014 was seen from the cause directly every year eclampsia was the biggest cause of 5 people, then bleeding as many as 3 people, and sepsis as many as 2 people.[5]

The purpose of this study was to determine differences in Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1) and Placental Growth Factor (PIGF) levels of early and late onset in preeclamptic women and normal pregnancy.

II. METHOD

The study was conducted at M. Djamil Hospital, Rasidin Hospital, Reksodiwiryo Hospital and Andalas University Biomedical Laboratory from July 2017-January 2018 and already approved by the study ethics committee of the Faculty of Medicine, Andalas University (Number 237 / KEP / FK / 2017). The sample of this study was all pregnant women from the age of 20 weeks to 40 weeks who came to M. Djamil Hospital, Rasidin Hospital, and Reksodiwiryo Hospital, diagnosed with preeclampsia based on clinical symptoms and laboratory examination results, a single pregnancy was determined from a doctor's examination seen in the record medical patient. Mothers with diabetes mellitus and active smokers will be excluded from this study.

Patients who meet the criteria and agree to participate will give informed consent and 3 cc of blood will be collected by the laboratory staff in the antenacubiti area, then the blood will be put into a vacutainer containing separating jelly and then centrifuged at 3000 rpm for 10 minutes.

The serum is stored at -80^{0}C until the examination is done at the Andalas University Molecular Biology Laboratory, Padang.

After testing the normality of the data with Kolmogrov-Smirnov after an unpaired T test was performed to determine differences ratio level Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1) and Placental Growth Factor (PIGF) levels of early and late onset in preeclamptic women and normal pregnancy.

III. RESULT

This study was conducted on 80 samples (20 respondents with early onset preeclampsia, 20 respondents with late onset diagnosis of preeclampsia, and 40 respondents with a diagnosis of normal pregnancy with the same gestational age as matching respondents diagnosed early and late onset of preeclampsia). The examination results can be seen in the table below.

Median Level of SFLT-1 in Study Subjects

In the initial process of testing the distribution of normal data, the Sflt-1 level showed an abnormal data distribution, therefore the data displayed was in the form of a median and the data was continued with the Mann-Whitney test. The Sflt-1 level of each group of study subjects can be seen in table 1 below:

Table 1Median Level of SFLT-1 in Study Subjects

Group	Levels Sflt-1 (ng/mL)			*P*
	Median	**Minimun**	**Maximum**	***value***
Early Onset				
Early Onset	8,69	5,69	14,80	**0,88**
Normal 1[a]	8,93	1,00	11,56	
Late Onset				
Late	9,33	5,15	12,37	**0,01***
Onset	8,56	7,31	9,33	
Normal 2[b]				
Preeclampsia				
Early Onset	8,69	5,69	14,80	**0,34**
Late Onset	9,33	5,15	12,37	

* there are significant differences

[a]gestational age<34 weeks

[b]gestational age ≥34 weeks

It can be seen that for the late onset group, there was a significant difference in mean SFLT-1 levels between late onset preeclampsia and normal pregnancy.

Average PlGF Level in Study Subjects

In the initial process of testing the distribution of normal data, PLGF levels showed normal data distribution, therefore the data displayed was in the form of data and continued with the Independent t test. PLGF levels for each group of study subjects can be seen in table 2 below:

Table 2 Average PlGF Levels in Study Subjects

Group	Levels PlGF (ng/mL)			*P*
	Mean	**SD**	**95% CI**	***value***
Early Onset				
Early Onset	6,57	1,19	-0,95	**0,30**
Normal 1[a]	6,25	0,71	– 0,30	
Late Onset				
Late Onset	6,24	0,65	-0,59	**0,63**
Normal 2[b]	6,12	0,83	– 0,36	
Preeclampsia				
Early Onset	6,57	1,19	-0,27	**0,27**
Late Onset	6,24	0,65	– 0,95	

[a] gestational age <34 weeks

[b] gestational age ≥34 weeks

Based on Table 2 it can be seen that for all groups there were no significant differences in mean PLGF levels between early onset and normal pregnancy, late onset with normal pregnancy, and early with late onset.

IV. DISCUSSION

During normal pregnancy proangiogenic conditions occur, namely the level of sFlt-1 is low until the end of the second trimester and PlGF levels are high. In preeclampsia when pregnancy increases, sFlt-1 levels

will gradually increase so that the balance will shift to weaken PlGF. Increased circulating PlGF and VEGF concentrations are low because they are bound by sFlt-1. This causes the process of placental angiogenesis to be disrupted.[6]

sFLT-1 is physiologically secreted by the human placenta and is produced in excess amounts by the placenta experiencing preeclampsia. sFLT-1 is the major endogenous inhibitor of angiogenesis found in the placenta.[6]

Differences in Median sFlt-1 levels between early onset and Normal

Median levels of sFlt-1 in the early onset group were 8.69 (5.69-14.80) ng / ml and the sFlt-1 level in normal pregnancy was 8.93 (1.00-11.56) ng / ml. Statistically it shows p value = 0.88, meaning that there is no significant difference between sFlt-1 level between early onset and normal pregnancy groups.

Similar results in other studies, in which pregnancy developed into early and late preeclampsia there was no significant difference between median levels of sFlt-1 compared to normal pregnancy (7099 (4769–58 270) vs 6840 (4200-11 381) vs 6349 (3697 –10 153) pg / ml; p = 0.736). Similar results were also found in other studies where there were no significant differences in sFlt-1 levels between early preeclampsia compared with the control group (0.88 (0.45–1.52) vs. 1.25 (0.62–1.80); p> 0.05).[7,8]

Different results were found by other studyers, where women with early onset preeclampsia had median sFlt-1 43 times higher than normal pregnancy (37,700 pg / ml vs. 886 pg / ml) with p <0.01. Different results were also found in subsequent studies, where the median sFLT-1 increased in value in women who became preeclampsia compared with normal pregnancies (1583 (1380–2532) vs. 1203 (1091-1290) pg / ml; p = 0.003).[9,10]

The difference between this study and previous study is likely to be due to the different study methods and characteristics of the sample used as respondents. In addition, in this study also found several maternal characteristic factors in the form of hemoglobin levels, leukocytes and hematocrit have levels that are statistically different between early onset and normal pregnancy. Therefore, the studyer argues that there is a possibility that one or more of these levels will have an effect on the onset of early onset which causes no difference in sFLT-1 levels between early onset and normal pregnancy. This study also did not assess Hb levels during pregnancy and BMI before pregnancy, where the two levels were considered to also affect the occurrence of preeclampsia.

In theory, weight can affect the occurrence of preeclampsia. Excess body weight before pregnancy increases the risk of PE. This is due to an increase in serum triglycerides and low lipoprotein levels in obese women. This lipid profile induces oxidative stress caused by ischemia, reperfusion mechanisms and neutrophil activation resulting in endothelial cell dysfunction. Besides that it causes atherothrombosis and induces platelet aggregation which ultimately causes coagulopathy which includes the characteristics of PE. A body weight that is less than normal is also at risk for PE. This is caused by a lack of protein that has an impact on immunological deficiency and reduced expression of proteins responsible for trophoblast invasion.[11]

Difference in Median Levels of sFlt-1 between Late Onset and Normal

Median levels of sFLT-1 in the late onset group were 9.33 (5.15-12.37) ng / ml and sFlt-1 levels in normal pregnancy were 8.56 (7.31-9.33) ng / ml. Statistically, it shows p = 0.01, meaning that there are significant differences in sFLT-1 levels between late onset and normal pregnancy groups.

Similar results were also found in other studies, in which women with late onset preeclampsia had median sFlt-1 3 times higher than normal pregnancy (26.106 pg /

ml vs. 7.827 pg / ml) with p <0.01. The same results were also obtained by subsequent studyers who found a median significant difference in sFLt-1 levels between cases with preeclampsia at ≥37 weeks' gestation with the control group (normal pregnancy) (1448 vs 1788 pg / ml; p = 0.002).[9,12]

Different results were found by different studyers who found that there was no significant median difference in sFlt-1 levels between the late preeclampsia group and normal pregnancy (6840 (4200–11 381) vs. 6349 (3697-10 153) pg / ml; p>0 , 05). Different results were also found by other studyers where there was no significant difference in median levels of sFlt-1 between late preeclampsia and the control group (0.98 (0.65–1.66) vs. 1.25 (0.62–1.80) ng / ml; p> 0.05). While other studies also found no difference between women with late onset preeclampsia and the control group both at 12-14, 18-20 or 26-28 weeks of gestation.[7,8,13]

Late onset preeclampsia is affected by extrinsic or maternal factors, where specific conditions that increase placental mass (diabetes or multiple pregnancies), or expand the placental surface (hypoxic condition of the mother, anemia), will cause excessive release of synctitial knots. Pregnant women with maternal risk factors, or with an inflammatory reaction that reacts inappropriately to the release of trophoblast apoptotic fragments can cause the clearing system to cope with an increased number of apoptotic fragments. Both of these ultimately lead to secondary necrosis in the blood which can cause late onset clinical symptoms.[9,14]

In this study maternal maternal factors were not found that could influence the late onset process so that the studyers argued that there was no difference in sFLT-1 levels between late onset and normal pregnancy, one of which was the presence of other factors that affected the work of sFLT-1. The exact cause of variations in results from several other studyers and the results of this study are still difficult to enforce. The differences in study design, subject characteristics, and examination method techniques carried out by other studies can provide results that are different from the results of current study.

Difference in Median Levels of sFlt-1 between early onset and late onset

Median levels of sFLT-1 in the early onset group were 8.69 (5.69-14.80) ng / ml and the level of sFlt-1 in the late onset group was 9.33 (5.15-12.37) ng / ml. Statistically, the p value = 0.34 means that there is no significant difference between sFLT-1 levels between early onset and late onset groups.

Different results were found by other studyers who found that levels of sFlt-1 in the early onset group of preeclampsia were higher than late onset preeclampsia (37,700 pg / mL vs 26,106 pg / mL). Different results were also found by subsequent investigators where the SFLT-1 level significantly increased in the early onset of preeclampsia compared to late onset preeclampsia and normal pregnancy.[9,15]

Early onset and late onset are two different clinical events that have different pathogenesis. early onset is caused by uteroplacental insufficiency in the early stages of pregnancy while late onset is more due to endothelial dysfunction caused by maternal factors. In general, signs and symptoms of maternal hypertension and proteinuria tend to be more severe in early onset. IUGR cases are also significantly higher in early onset compared to late onset. Previous studies have shown that early onset is usually associated with IUGR, causing poor pregnancy outcomes. Conversely, late onset is most often associated with moderate-level maternal symptoms that have a mild effect on the fetus.[15]

Other studyers also found that serum sFLt-1 was significantly higher in women who would later experience early onset of preeclampsia than women who would develop late onset preeclampsia and compared controls with and without risk

factors at 26-28 weeks gestation (4847.3 (1318.6 -17819.7) vs 1054.4 (887.2-1252.9) 1054.4 (887.2-1252.9); p <0.05).[13]

In this study there were no differences in sFLT-1 levels between early onset and late onset although there were several levels of blood parameters (hemoglobin, leukocyte and platelet levels) which were statistically different in the two groups of preeclampsia compared with normal pregnancy. Studyers argue that some of these factors can affect the onset of early onset or late onset. The exact cause of variations in results from several other studyers and the results of this study are still difficult to enforce.

Average PlGF Level between Early Onset and Normal

The average level of PlGF in the early group onset was 6.57 ± 1.19 ng / ml and the level of PlGF in normal pregnancy was 6.25 ± 0.71 ng / ml. Statistically it shows p value = 0.30, meaning that there is no significant difference between PlGF levels between early onset groups and normal pregnancy.

Similar results were found by a studyer where there was no difference in PlGF levels in the early, late and normal groups at 20 weeks' gestation (136.3 ± 109.5 vs 228.8 ± 150.2 vs. 272.6 ± 169.8 ; p = 0.045).[16]

Different results were found by other studyers in which PlGF concentrations were found to be lower in early pregnancy with preeclampsia compared with normal pregnancies (31 ± 12 pg / ml vs 39 ± 32 pg / ml) with a value of p = 0.01.[17]

Different results were also found by other studyers when compared with the control group, serum PlGF levels were lower than women who had preeclampsia (23 ± 24 pg / ml vs. 63 ± 145 pg / ml; P <0.01) or gestational hypertension (27 ± 19 pg / ml; P = 0.03), or who gave birth to a baby with IUGR (21 ± 16 pg / ml; P <0.01).[18]

PIGF is an important amino acid glycoprotein dimer residue in local mediators in the process of angiogenesis. PIGF is produced by the placenta mainly by the cytotrophoblast, syncytiotrophoblast and extra filament trophoblasts. PIGF is also produced in human umbilical venous endothelium and cariocarcinoma cells. Changes in the concentration of PIGF circulation can affect the balance of placental angiogenesis. This condition usually occurs in patients with preeclampsia because it reflects abnormalities in placental development.[19]

The difference between this study and previous study is probably due to the fact that in this study some maternal characteristic factors were found in the form of hemoglobin levels, leukocytes and hematocrit had levels that were statistically different between early onset and normal pregnancy. Therefore, the studyer argues that there is a possibility that one or more of these levels will have an effect on the onset of early onset which causes no difference in sFLT-1 levels between early onset and normal pregnancy. This study also did not assess Hb levels during pregnancy and BMI before pregnancy, where the two levels were also considered to affect the occurrence of preeclampsia.

Average PlGF Level between Late Onset and Normal

The mean PlGF level in the late onset group was 6.24 ± 0.65ng / ml and the PlGF level in normal pregnancy was 6.12 ± 0.83 ng / ml. Statistically, the p value = 0.63 means that there is no significant difference in PlGF levels between the late onset group and normal pregnancy.

The same results were found by a studyer who did not find any differences in preeclampsia with controls with or without risk factors at 28 weeks' gestation (274.2 (222.4-338.1) vs. 271.5 ‡ (224.9-327.6) vs 383.8 (332.3-443.3); p> 0.05). Then the same results were also found by other studyers where late onset PlGF levels were slightly reduced compared to normal pregnancy although there were no significant differences between the two groups.[13,15]

Different results were found by a studyer who found a significant difference in PlGF log levels between the late and control groups (1.77 ± 0.28 vs 2.58 ± 0.32; p <0.0001). Different results were also found by other studyers where PlGF is one of the biomarkers that is considered to have high sensitivity to predict the occurrence of late onset.[21,12]

Late onset placental examination shows that substantially fewer pathological lesions occur. The closer to normal delivery time, the signs of symptoms occur the fewer the number of pathological lesions that occur. Factors of angiogenic and antiangiogenic disorders are related to various clinical pathologies of the mother and fetus. Antiangiogenic factors are the most common in early onset. Mild disorders that may cause other conditions to occur later, including premature labor or premature rupture of membranes. For the onset of early onset, placental ischemic lesions that occur must cover a large area, if not wide enough it is likely to cause premature or late onset labor. This may be attributed to the presence of a placental defense mechanism.[21]

In this study found maternal characteristic factors in the form of leukocyte levels that have levels that are statistically different between late onset and normal pregnancy. Therefore, the studyers argue that in addition to the causes mentioned above, there is a possibility that one or more of these levels will have an effect on the onset of late onset which results in no difference in late-onset and normal pregnancy.

Rerata Kadar PlGFantara*Early Onset*dengan *Late Onset*

The mean PlGF level in the early onset group was 6.57 ± 1.19ng / ml and the late onset PlGF level was 6.24 ± 0.65ng / ml. Statistically it shows p value = 0.27, meaning that there is no significant difference in PlGF levels between early onset groups with late onset.

The same results were found by a studyer where PlGF levels were found to decrease in early onset compared to late onset and normal pregnancy. PlGF levels in the early onset were slightly reduced compared to normal pregnancy but there were no differences between the two groups.[15]

Different results were also found by other studyers where there were differences in PlGF levels between the early and late preeclampsia groups (258.9 ± 142.9 vs. 424.1 ± 208.5 pg / ml; p = 0.0003). Then different results were also found by other studyers where serum PlGF decreased in levels compared to late (24.9 (15.7-48.4) vs. 59.4 (33.3-190.0); p = 0.004). While other studyers also suggested that the levels of PlGF tended to rise with the age of pregnancy.[16,20,21]

Both early onset and late onset show similar signs of clinical symptoms and conditions that are generally the same as angiogenesis, inflammation and endothelial damage have been shown to exist. However, some studyers believe that despite many similarities, they are different entities. In early onset, trophoblast invasion disorders in the early stages of trophoblast development are the dominant causative factors. This is seen in the disruption of flow in the uterus and umbilical cord which is most common in early onset. Late onset, although impaired spiral artery conversion affects but is less significant than early onset. late onset is more due to the constitutional characteristics of pregnant patients themselves such as insulin resistance or metabolic syndrome that determine the development of preeclampsia.[21,22,23]

Placental hypoperfusion occurs both in early onset and late onset although signs of symptoms are usually milder in late onset. Disorders of angiogenesis markers such as sFLT1 and PlGF have been shown to play a significant role in the occurrence of preeclampsia. It has been found that PiGF levels are much lower in cases suspected of having placental hypoperfusion as

evidenced by histopathological tests. PIGF levels are definitely lower in early onset. Low PlGF levels have also been shown to be associated with serious pathology in the placenta. Treatment with PlGF shows a reduction in symptoms of preeclampsia.[24,25]

In this study there were no differences in PlGF levels between early onset and late onset although there were several levels of blood parameters (hemoglobin, leukocyte and platelet levels) which were statistically different in the two groups of preeclampsia compared with normal pregnancy. Studyers argue that some of these factors can affect the onset of early onset or late onset.

V. CONCLUSION

There is a difference in Soluble Fms-Like Tyrosine levels late onset kinase-1 in preeclamptic women and normal pregnancy. This shows that there is a high likelihood of sFlt-1 mothers being at high risk of developing late onset preeclampsia.

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