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High level of soluble FMS-like Tyrosine Kinase-1 (sFlt-1) serum in pregnancy as a risk factor of preeclampsia

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ABSTRACT

Background: Recently, etiology and pathogenesis of preeclampsia remain unknown. One of the theory indicating that hypoxia and ischemic placenta caused by abnormal cytotrophoblast invasion in preeclampsia. Soluble FMS-like tyrosine kinase-1 (sFlt-1) serum as a laboratory marker of hypoxia condition that contributes to the occurrence of endothelial damage and clinical manifestations in preeclampsia.

Objective: This study was aimed at proving that high level of soluble FMS-like tyrosine kinase-1 (sFlt-1) serum in pregnancy as a risk factor for preeclampsia.

Methods: This study was a case control. Among 58 pregnant women studied, 29 women with preeclampsia as a case group and 29 women with normal pregnancy as a control group. Soluble FMS-like tyrosine kinase-1 (sFlt-1) serum was analyzed in the Prodia Laboratory. Collected data were tested for normality using Kolmogorov-Smirnov, then analyzed with independent sample test. Chi-Square test used to determine soluble FMS-like tyrosine kinase-1 (sFlt-1) serum level in preeclampsia.

Results: This research concluded that the average level of soluble FMS-like tyrosine kinase-1 (sFlt-1) serum in preeclampsia were 11231.00 ± 8390.3 pg/mL and 3981.62 ± 4921.5 pg/mL in normal pregnancy. Analysis of significance with independent t-test concluded that the value of t = 4.01 and p = 0.001. This mean the average levels of soluble FMS-like tyrosine kinase-1 (sFlt-1) serum levels in both groups were difference significantly (p <0.05). Based on the cut-off point of sFlt- 1 serum levels was 4505.50 pg/mL with 79.3% sensitivity and 82.8% specificity, the relative risk of preeclampsia was 18 times (OR = 18.40, IK 95% = 4.93 to 68.70, p = 0.001).

Conclusion: Based on this research, high levels of soluble FMS-like tyrosine kinase-1 (sFlt-1) in pregnancy was proved as a risk factor for preeclampsia.

Keywords: preeclampsia, soluble FMS-like tyrosine kinase (sFlt-1).
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INTRODUCTION

Preeclampsia is one of leading cause of maternal and fetal mortality and morbidity worldwide. It's prevalence in the world has reported around 3-8%. WHO reported that 18% of maternal mortality and 40% of fetal mortality are caused by preeclampsia.[1]

In Indonesia (2005), the prevalence of maternal mortality caused by preeclampsia and eclampsia was reported around 4,91%, the highest prevalence among South East Asia's countries.[2]

The common risk factors of preeclampsia are divided into many groups: (1) Primigravida (3-10%); (2) Hyperplacentosis, such as mola hydatidosa, multifetal pregnancy, diabetes mellitus, macrosomia, (13%); (3) Maternal age less than 20 yaers (38%) and more than 35 years (37,6%); (4) Family history of preeclampsia or eclampsia; (5) Kidney disease and chronic hypertension; (6) Race (Afro-American 3-10%); (7) obesity (13%).[1,3]

However, clinical diagnosis and definition of preeclampsia is commonly based on the measurement of nonspecific signs and symptoms, principally proteinuria and hypertension.[4] In the decade, some specific proteins have established as predictor markers to diagnose preeclampsia. One of those markers were soluble FMS-like tyrosine kinase (sFlt-1) which can be detected in the second and third trimester of pregnancy with specificity and sensitivity around 80-90%.[5]

The excessive placental production of sFlt-1, an antagonist vascular endothelial growth factor (PIGF), contributes to the pathogenesis of ischemic placental in preeclampsia and eclampsia. Various reports have demonstrated that disturbance in angiogenic and antiangiogenic factors are implicated in the pathogenesis of preeclampsia and have possible relevance in the diagnosis of the disease.[6,7]

In 2014, extensive research conducted by Levine et al[7] reported a significant difference between level of sFlt-1 in preeclampsia (± 4382 pg/ml) and normal pregnancy (± 1643 pg/ml). Study by Anderson

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et al[8] also showed the same result, demonstrating the usefulness of angiogenic markers in both diagnosis and prediction of preeclampsia and placental disorder. Moreover, this study was aimed at proving that high level of sFlt-1 serum in pregnancy is a risk factor for preeclampsia.

MATERIAL AND METHOD

Study Design and Sample Collection

A case control observational study was conducted at Obstetrics and Gynecology Department of Sanglah General Hospital from December of 2013 until January of 2015. Pregnant women with preeclampsia at 20–30 weeks of gestational age who agreed to participate were selected. Pregnant women with comorbidities such as diabetes mellitus, chronic hypertension, renal disease, and multifetal pregnancy were excluded from the samples. According to sample calculation, the minimum number of sample required was 29 pregnant women without preeclampsia (control group) and 29 pregnant women in preeclampsia group. Total sample needed for analytical purpose was 58 subjects, divided into 2 groups. 5 ml of peripheral venous blood were obtained and collected in EDTA tube until processed further.

Blood Processing and Serum Collection

After blood samples were collected, the blood was processed in EDTA tubes. All of the blood samples were centrifuge within speed 1300 – 2000 rpm for 15 minutes to separate between serum and supernatants. Then the serum was transferred into the kit, incubating at 4°C for 1 week. If the serum and control solution was stabile after 1 week, it will be analyzed by the reagent solution at -20°C.

Statistical Analysis

All of the data analysis was conducted by SPSS version 17. Baseline characteristic of the samples will be analyzed descriptively and listed in table 1. Then, normality homogeneity test was conducted to determine data distribution. Independent T-test procedure was used to compare means of sFlt-1 level in preeclampsia group and control group. The association between sFlt-1 level with preeclampsia in pregnancy was analyzed by *Chi-square* test. To evaluate the diagnostic value of sFlt-1 as predictor of preeclampsia in pregnancy, we calculate sensitivity, specificity, likelihood ratio and predictive value of sFlt-1.

RESULTS

The mean age of subjects was 28.28 years for preeclampsia group and 28.10 years for control group. There are no significant differences in gestational age as well as number of parities of the samples. Normality assessment of the data showed that the data were normally distributed.

Table 1 **Baseline Characteristic of Subjects**

Baseline Character	Group		*p*
	Preeclampsia	Control	
Maternal Age (years)	28,28±7,78	28,10±5,41	0,922
Gestational Age	35,69±3,19	33,76±4,15	0,052
Gravida	0,91±0,84	0,82±0,86	0,450

Table 2 **Level of sFlt-1 in Preeclampsia and Normal Pregnancy**

Group	N	Level of sFlt-1 (mean ± SD)	P
Pre-eclampsia	29	11231 ± 8390,3	0,001
Control	29	3981,62 ± 4921,5	

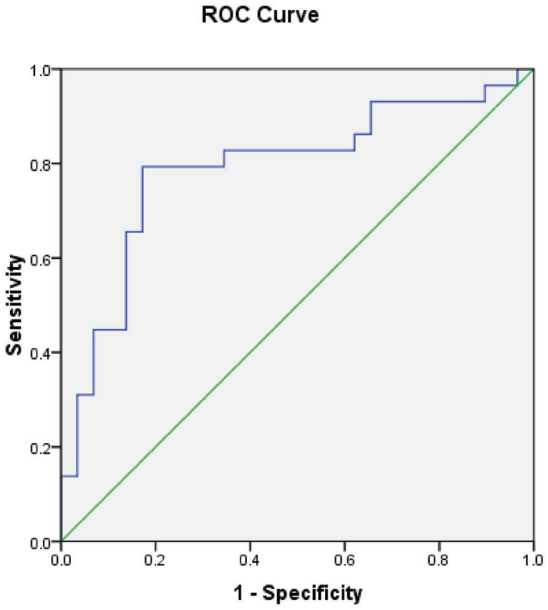

Figure 1 ROC curve shows the specificity and sensitivity of sFlt-1 in Preeclampsia.

Independent *T-test* was performed to analyze the difference between the mean of sFlt-1 level in Pregnant women with preeclampsia and normal pregnancy. The result of this test showed a significant difference between level of sFlt-1 in preeclampsia women ($p< 0,05$).

Cross tabulation of sFlt-1 level and preeclampsia was conducted to show its association with preeclampsia. *Chi-square* test showed that high level of sFlt-1 was significantly associated with preeclampsia ($p<0.05$) and high level of sFlt-1 serum will increase the risk of preeclampsia 18 fold (OR=18,40, IK 95% = 4,93-68,70, p = 0,001).

Diagnostic analysis of the data used ROC kurve showed that cut off point of the level of sFlt-1 was 4505,50 pg/mL with 79,3% sensitivity and 82,8% specificity (Figure 1).

DISCUSSION

Preeclampsia is a challenge for health care provider, due to the unspecific marker and unclear risk factor predictor. This study supports the suggestion obtained in many studies conducted in several countries that preeclampsia is associated with a disturbance between pro- and antiangiogenic factors. A research conducted by Andersen et al. (2014) showed the cut-off point of sFlt-1 level in preeclampsia was 6570.60 pg/mL, while the levels of sFlt-1 in normal pregnancy was 3553.42 pg/mL (p<.001). Moreover, Hasan et all also obtained a consistent result with Andersen. The sensitivity of sFlt-1 as a risk factor of preeclampsia was 88% while the specificity was 83.6% and accuracy 84.7% with an odds ratio (OR) 37.2 [95 % confidence interval (CI) 17.7 to 78.1].

Based on the ROC analysis we found the cut-off point of sFlt-1 level between preeclampsia and normal pregnancy was 4505.5 pg/mL. Furthermore, in this study we also found the sensitivity sFlt-1 as a predictor risk of preeclampsia was 79.3% and the specificity was 82.8%. To determine the risk of preeclampsia in the mothers with high level of sFlt-1 we used *chi-square* analytical test. It showed the odds ratio levels of soluble sFlt-1 is high (OR = 18.40, 95% CI = 4.93 to 68.70, p = 0.001). It's mean high level of sFlt-1 in pregnancy will increase the risk of preeclampsia 18 fold at the 20 – 30 weeks of gestational age.

The difference among study designs, gestational age, sample collections, baseline characteristic of the subjects (race and obesity), sample population, and sample examination method affected the varieties of the results in current study and another studies before. Association of obstetrics and gynecology noted that Afro-Americans race develop preeclampsia by 3-10%, but there is no theory can explain the pathophysiology of this. Another risk factor of preeclampsia was obesity. Maternal obesity will increase the risk of preeclampsia 4,3% in women with *Body Mass Index* (BMI) < 20 kg/m^2 and increase the risk of preeclampsia 13,3% in women with BMI > 35 kg/m^2.[11]

Maternal obesity leads to lipotoxic condition characterized by decreasing angiogenic regulators and increasing pro-inflammatory cytokines. Obesity increases production of monocyte chemoattractant protein-1 (MCP-1) in the placenta, followed by huge number macrophages infiltration in the placenta. Circulating macrophages produce larger quantities of pro-inflammatory cytokines such as IL- 1 and TNF-α. The pro-inflammatory cytokines especially TNF-α through the process of apoptosis can limit the invasion of cytotrophoblast cells to the spiral arteries. Interestingly, the decidua macrophages express the Flt-1 receptor. While macrophages isolated from the placenta in the first and third trimester are naturally secreting low level of sFlt–1. Presence of lipopolysaccharides will increase the secretion of sFlt-1 4 times higher than normal condition.[22]

The failure invasion of cytotrophoblast will decrease the diameter of spiral arteries. Furthermore, if the pressure of spiral arteries is too high accompanied by acute atherosis will induce an endothelial damage, necrosis, and accumulation of foam cells. The impact of endothelial damage and accumulation of foam cells was blocking of the spiral arteries. This condition cause hypoxia in the placental environment. These were then triggered to release some markers of placental hypoxia. One of the marker was sFlt – 1, which is increasing sFlt-1 secretion will release the sFlt-1 serum into the systemic circulation of the mother. Placental perfusion deficiency was the leading cause of ischemic perfusion injury in the placenta, so that all of these process was causing symptoms of preeklampsia.[21]

CONCLUSION

SFlt-1 serum is a laboratory marker of hypoxia condition that contributes to the occurrence of endothelial damage and clinical manifestations in preeclampsia. Our study confirmed that high levels of soluble FMS-like tyrosine kinase-1 (sFlt- 1) in pregnancy was proved as a risk factor for preeclampsia. It increases the risk of preeclampsia 18 fold at the 20 – 30 weeks of gestational age. However, further study is needed in order to confirm it diagnostic value.

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