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Original Article

THE ROLE OF PREDICTOR PLACENTAL GROWTH FACTOR, SOLUBLE ENDOGLIN, SOLUBLE-FMS-LIKE-TYROSINE KINASE-1 AND PULSATIL UTERINA ARTERIAL INDEX TO PREDICT THE EARLY ONSET OF PREECLAMPSIA

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ABSTRACT

Objective: In preeclampsia, there will be an increase in SFlt-1 and in and decrease in PlGF levels. This condition will cause disorders of vasculogenesis and angiogenesis in fetomaternal circulation, which will eventually lead to preeclampsia syndrome such as proteinuria, hypertension and endothelial dysfunction.

Methods: An observational study design with nested case-control. The study was conducted at Bunda Thamrin Hospital, Tanjung Mulia Medika Hospital, Sundari Hospital and private practice, from March to November 2018, with a sample of 64 research subjects.

Results: The results of this study indicate that there were significant values with p<0.05, namely the pulsatile value of the uterine artery index with a 1.228 cut-off point, Area Under Curve (AUC) of 78.2% (95% CI 59.3%-97%), sensitivity 80%, specificity 64.6%, PlGF level with 441 pg/ml cut-off point, Area Under Curve (AUC) of 82.5% (95% CI 61.5%-100%), sensitivity 80%, specificity 87.7%, sFlt-1 level with a cut-off point of 10087.5 pg/ml, Area Under Curve (AUC) of 81.2% (95% CI 63.6%-98.9%), sensitivity 80%, specificity 67.7% while sEng with p value>0.05 which means it is not significant.

Conclusion: From this study, no significant differences were found in sEng, whereas differences were found in the pulsatile value of the uterine artery index, PlGF levels, and sFlt-1 levels in the incidence of early-onset preeclampsia. From the multivariate analysis, an examination of PlGF levels alone is sufficient as a predictor of early-onset preeclampsia.

Keywords: Preeclampsia, PlGF, sFlt1, Pulsatil Index, sEng

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INTRODUCTION

One form of hypertension in pregnancy is preeclampsia, which is characterized by hypertension with blood pressure ≥ 140/90 mmHg and protein in the urine at gestational age after 20 w. Preeclampsia is a global problem affecting 2-8% of pregnancies, and an estimated 8.3 million pregnant women experience preeclampsia every year. In developing countries, the priority is to prevent maternal death due to multiorgan complications [1]. In developing countries, there were 13 cases of preeclampsia in every 1,000 births, whereas in developed countries, only 2-3 cases of preeclampsia were found in every 10,000 deliveries [2, 3].

One of the theories of the pathogenesis of preeclampsia is that it is thought to be related to the failure of cytotrophoblast cells to invade the maternal spiral arteries, causing vascular injury and placental ischemia. As a result of placental ischemia, anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) are secreted, so that endothelial blood vessels are inhibited in carrying out angiogenesis. As a result of placental ischemia, endothelial dysfunction occurs. Endothelial dysfunction causes progressive tissue and multiorgan damage of the mother and fetus. sEng is the soluble form of a surface growth co-receptor transforming growth factor (TGF-β1 and TGF-β3) expressed in endothelial cells and syncytiotrophoblasts. It modulates the work of TGF-β1 and TGF-β3, which play an important role in blood vessel homeostasis. Concentration increased when placental perfusion is poor so that levels of sEng can act as a marker for changes in the impedance of uteroplacental circulation [3, 4].

In preeclampsia will be found an increase in sFlt-1 and sEng levels and a decrease in PlGF levels. This situation will cause vascularity and angiogenesis disorders in the fetomaternal circulation, which will eventually lead to preeclampsia syndromes such as proteinuria, hypertension and endothelial dysfunction. Because an imbalance between angiogenic and antiangiogenic factors has been observed when the diagnosis of preeclampsia is established, it can sometimes even be seen before clinical symptoms appear. Then angiogenic factors such as PlGF and antiangiogenic factors such as sFlt-1 and sEng can be a screening or assessment of risk factors for preeclampsia [5].

In a normal pregnancy, the pulsating index (PI) and resistance index (RI) values will decrease after 24-26 w of pregnancy, so that a permanent picture is formed, which is a picture of high and almost horizontal diastolic velocity. The picture of uterine artery waves in the first trimester of pregnancy has a winding diastolic peak (diastolic notch) that disappears after 24 w of pregnancy. If this indentation is permanent and the PI and RI values remain high after 20-24 w' pregnancy, it means that there is high pressure on the uterine arteries, which usually results in preeclampsia or stunted fetal growth [6].

Uterine artery velocimetry doppler examination to predict the incidence of preeclampsia is better done in the second trimester compared to the first trimester. The pulsality index with the diastolic notches examined in the second trimester obtained a pulsality index with a positive like hood ratio of 4.5. While the positive likelihood value of the resistance index is 3.5.6 Meanwhile, according to previous research, it was shown that prevention of preeclampsia by examination of sFlt-1, sEng and PlGF levels in maternal blood is best done in the second trimester because the invasion of the spiralsal trophoblast has been completed [5].

MATERIALS AND METHODS

This type of research is analytical research with a nested case-control study design conducted from March-November 2018 at Bunda Thamrin Hospital, Mitra Medika Tanjung Mulia Hospital, Sundari Hospital and private practice. The sample of this study were

normal pregnant women with 22-24 w gestation who came to the Obstetric Polyclinic with each group of 35 people for positive uterine notches (+) and 35 people for negative uterine notches (-) who met the inclusion criteria ie willing participated in the study and signed informed consent, pregnant women with 22-24 w gestation, live fetuses, single or multiple fetuses and exclusion criteria, namely anomalies found in the fetus, pregnant women with chronic hypertension, pregnant women with gestational hypertension, and have diseases which requires the termination of pregnancy before time. The research sample will be chosen by a non-random selection method, namely by consecutive sampling technique.

Work arrangement

After obtaining approval from the ethics commission to conduct research, data on research subjects were obtained, asked about the First Day of Last Menstruation, menstrual cycles and complaints during pregnancy. Furthermore, weight checks using digital scales, height using microtoise, vital signs (blood pressure, pulse, breathing frequency and body temperature), obstetrics, urinalysis lab, routine ultrasound and examination of uterine artery doppler.

The blood flow spectrum on Doppler is analyzed by video, using an electronic gauge to measure the systolic peak, the diastolic end and the average blood flow velocity. The pulsatile index is obtained from the mean of 3 consecutive waves. Abnormal uterine artery velocimetry Doppler means that of 3 consecutive consistent waves found notches in either the unilateral or bilateral uterine arteries and/or the mean pulsatile index>1.45 (Yusrawati, 2013).

Both groups had blood drawn from the venous mediana cubital vein and put into a vacuum tube containing EDTA to be sent to the laboratory. Blood samples were taken during the first visit and sent to the Prodia Laboratory in Medan. Increases or decreases in sFlt-1, sEng and PlGF levels are assessed from the tachycdiastolic group by comparing the levels of sFlt-1, sEng and PlGF in the group without tachycdiastolic.

Both groups were followed until the third trimester and patients were scheduled for every 4 w until 33 w 6 d gestation. At the next visit, the patient will be examined for weight, height, vital signs and obstetric examination. If there is an increase in blood pressure ≥ 140/90 mmHg and proteinuria≥300 mg in urine 2 4 h or on 2 measurements using a urinalysis lab with+1 results or 1 measurement using a urinalysis lab with results 2+2, then the patient is said to have preeclampsia. Blood pressure is determined based on an average of 2 or more examinations at different times and measurements are taken in a sitting position. After all, data is obtained, the data is processed and analyzed statistically.

Statistical analysis

Data on the characteristics of research subjects will be arranged in a frequency distribution table. On the bivariate data, a normality test will be conducted using the Kolomogorov-Smirnov test. If the normality test results obtained $p<0.05$ means the data is not normally distributed, then the data will be analyzed using the Mann-Whitney or Fisher exact test. If the data is normally distributed, then the data analysis will use the t-test or chi-square. Data analysis using SPSS version 22. In this study, the type of multivariate analysis used was multiple logistic regression to determine the variable with the best statistical value.

RESULTS

Characteristics of research subjects

The study was attended by as many as 70 pregnant women with 22-24 w gestation who came to the Obstetric Polyclinic of Bunda Thamrin General Hospital, Mitra Medika Tanjung Mulia Hospital, Sundari Hospital and private practices that met the inclusion and exclusion criteria. Subjects with 24 w gestational age were the most subjects with 35 people (50%). A total of 31 subjects (44.3%) were primigravidas

Table 1: Characteristics of research subjects

Characteristics	n = 70
Gestational age, n (%)	
22 w	22 (31,4)
23 w	13 (18,6)
24 w	35 (50)
BMI, Mean (SD), kg/m2	24,47 (4,02)
Parity, n (%)	
Primigravida	31 (44,3)
Secundi Gravida	23 (32,9)
Multigravida	16 (22,9)

Pulsatile index and diastolic notches of right and left uterine artery examination results

The results of the right and left uterine artery pulsatile index are presented in full in table 2. The mean index of the right uterine artery pulsatile is 1.09 with SD = 0.38, with the lowest value of 0.4 and the highest of 2.08. Meanwhile, the left uterine artery pulsatility index obtained a mean value of 1.18 with an SD value of 0.51, with the lowest value of 0.39 and the highest of 2.75.

Table 2: Pulsatile index of right and left uterine artery examination results

	Right uterine artery	Left uterine artery
Mean	1,09	1,18
SD	0,38	0,51
Minimum	0,4	0,39
Maximum	2,08	2,75
95% IK	0,99–1,18	1,06–1,30

In table 3. By using ultrasound, it is known that as many as 50% of subjects have without diastolic notches. A total of 27 subjects (38.6%) had unilateral tachycarastolic and 8 subjects (11.4%) had bilateral tachycdiastolic.

Table 3: Results of diastolic notches of right and left uterine artery examination

Diastolic notches, n (%)	n = 70
None	35 (50)
Diastolic Notches Unilateral	27 (38,6)
Diastolic Notches Bilateral	8 (11,4)

The results of the examination of uterine artery pulsatile index(PI), PlGF, sEng, sFlt-1 levels are presented in full in table 4. The mean uterine artery PIvalue was 1.14, with the lowest value of 0.44 and the highest of 2.08. The mean PlGF was 834.21 with SD = 413.75, with the lowest value of 165 and the highest of 2097. In measuring sEng, the average value of 5.37 was obtained with an SD value = 1.99, with the lowest value 2.66 and the highest 12.68. And the results of sFlt measurements showed an average of 10,303.31 with SD = 7544.99, the lowest level was 1554 and the highest was 47236.

Early onset preeclampsia

From the results of monitoring of all subjects during the study found that there were 65 subjects (92.9%) did not experience preeclampsia, 2 subjects (2.9%) had preeclampsia (proteinuria+3) and 3 subjects (4.3%) with preeclampsia (proteinuria+4).

Table 4: Uterine artery PITest results, placental growth factor (PlGF), soluble endoglin (sEng) and soluble-fms-tyrosine kinase (sFl-1) levels

	PIA. Uterina	PlGF	sEng	sFlt
Mean	1,14	834,21	5,37	10303,31
SD	0,36	413,75	1,99	7544,99
Minimum	0,44	165	2,66	1554
Maximum	2,08	2097	12,68	47236
95% IK	1,05-1,22	735,56-932,87	4,90–5,85	8504,27-12102,36

Table 5: Incidence of early onset preeclampsia based on proteinuria

Early onset preeclampsia	n = 70
Normal	65 (92,9)
Preeclampsia (Proteinuria+3)	2 (2,9)
Preeclampsia (Proteinuria+4)	3 (4,3)

Differences in uterine artery PIValue, PlGF, sEng, sFlt-1 levels

Average PI uterine artery in the group of subjects with diastolic notches was 1.42 (SD = 0.26) while the mean PI uterine artery in the group of subjects without diastolic notches was 0.85 (SD = 0.17). Using the Independent T test shows that there are differences in the average PIa. Significant erythema between subjects with diastolic notches and without diastolic notches (p<0.0001).

Table 6: Differences in uterine artery PI values, PlGF, sEng, sFlt-1 levels between subjects with uterine artery diastolic notches and without diastolic notches

	Diastolic notches		P value
	+(35)	-(35)	
PIa. Uterina, mean (SD)	1,42 (0,26)	0,85 (0,17)	<0,001a
PlGF, mean (SD)	695,6 (385,69)	972,83 (398,88)	0,004a
sEng, mean (SD)	5,44 (2,39)	5,30 (1,51)	0,533b
sFlt-1, mean (SD)	11907,91(8454,01)	8698,71 (6225,52)	0,006b

[a]T Independent, [b]Mann Whitney

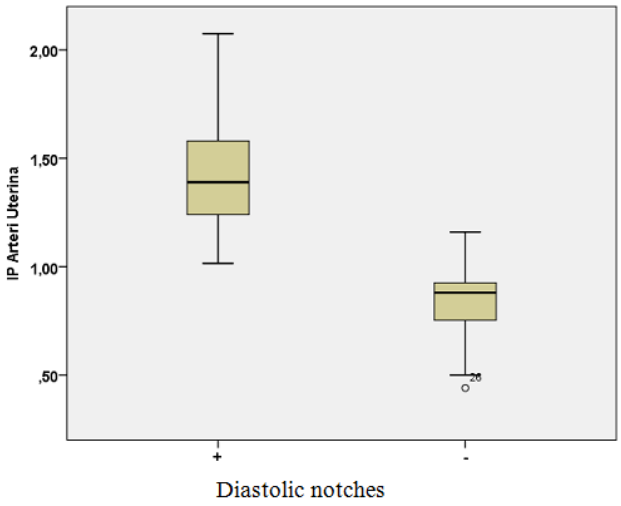

Fig. 1: Boxplot graph difference of piuterine artery in subjects with diastolic notches and without diastolic notches

The mean PlGF level in the group of subjects with diastolic notches subjects was 695.6 (SD = 385.69) while the mean PlGF level in the group of subjects without diastolic notches was 972.83 (SD = 398.88). Using the Independent T test showed that there was a significant difference in mean PlGF between subjects with diastolic notches and without diastolic notches (p = 0.004).

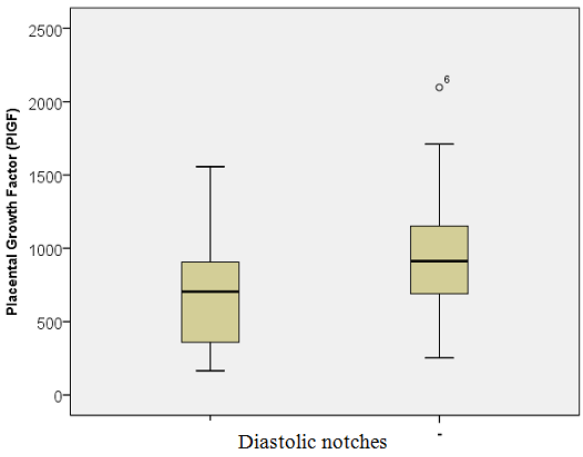

Fig. 2: Boxplot graph difference of PIGF levels in subjects with diastolic notches and without diastolic notches

The mean sEng level in the group of subjects with diastolic notches was 5.44 (SD = 2.39) while the mean sEng in the group of subjects without diastolic notches was 5.30 (SD = 1.51). Using the Mann Whitney test showed that there was no significant mean difference between subjects with diastolic notches and without diastolic notches (p = 0.533).

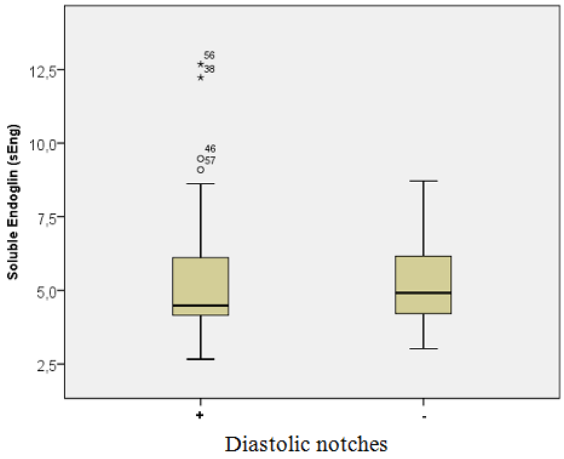

Fig. 3: Boxplot graph difference of sEng levels in subjects with diastolic notches and without diastolic notches

The mean level of sFlt-1 in the group of subjects with diastolic notches was 11907.91 (SD = 8454.01) while the mean sFlt-1 in the group of subjects without diastolic notches was 8698.71 (SD = 6225.52). Using the Mann Whitney test showed that there were significant differences in sFlt-1 between subjects with diastolic notches and without diastolic notches (p = 0.006).

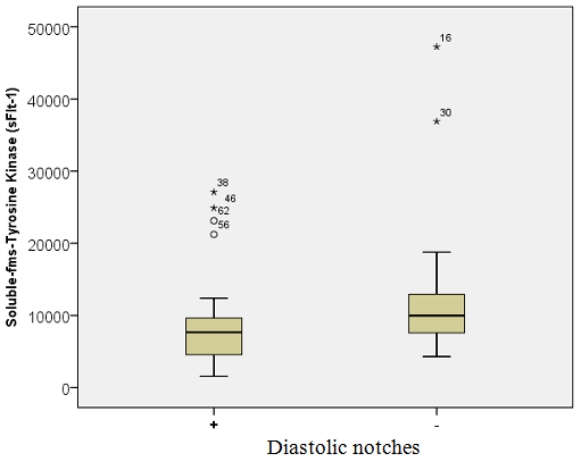

Fig. 4: Boxplot graph difference of sFlt-1 Levels in subjects with diastolic notches and without diastolic notches

Relationship of age, parity, BMI, tachycardastolic, uterine artery pulsatile index, PlGF, sEng, sFlt-1 to early-onset preeclampsia

There were no subjects older than 35 y who had early onset preeclampsia, while there were 5 people (8.1%) subjects who were ≤35 y old had preeclampsia. The results of the analysis using the Fischer's exact test showed that no significant relationship was found between age and the incidence of early onset preeclampsia (p = 1,000).

Of the 31 subjects with primigravida pregnancy there were only 3 subjects (9.6%) who had early onset preeclampsia, while there were 2 more samples with early onset preeclampsia found in mothers with multigravida pregnancy. The results of the analysis using the Fischer's exact test showed that no significant relationship was found between maternal parity and the incidence of early onset preeclampsia (p = 0.251).

Of the 32 subjects who had BMI overweight and obesity there was only 1 subject (3.1%) who had early onset preeclampsia, while there were 4 people (14.8%) subjects with underweight and normoweight who had preeclampsia.

The results of the analysis using the Fischer's exact test showed that no significant relationship was found between BMI and the incidence of early-onset preeclampsia (p = 0.169).

Of the 35 subjects who had uterine artery diastolic notches, there were 5 subjects (14.3%) who had early-onset preeclampsia, while no preeclampsia was found in subjects who did not have diastolic notches. The results of the analysis using Fischer's exact test showed that no significant relationship was found between uterine artery diastolic notches and the incidence of early onset preeclampsia (p = 0.054).

Average PIuterine artery in subjects with early onset preeclampsia was seen to be higher with a mean of 1.44 (SD = 0.30) than subjects without preeclampsia with a mean of 1.11 (0.35). Using the Independent T test showed that there were differences in the mean PlGF levels between subjects with preeclampsia and subjects without preeclampsia (p = 0.045).

The mean PlGF in subjects with early onset preeclampsia was seen to be lower with the mean of 411 (SD = 301.67) compared to subjects without preeclampsia with a mean of 866.77 (404.73). Using the Mann Whitney test showed that there were differences in the mean PLGF levels between subjects with preeclampsia and subjects without preeclampsia (p = 0.016).

The mean sEng in subjects with early onset preeclampsia was seen to be higher with mean 8 (SD = 4.11) than subjects without preeclampsia with a mean of 5.17 (1.62).

Using the Mann Whitney test showed that there was no difference in mean levels between subjects with preeclampsia and subjects without preeclampsia (p = 0.113).

The mean sFlt-1 in subjects with early-onset preeclampsia was seen to be higher with a mean of 17943 (SD = 8346.99) than subjects without preeclampsia with a mean of 9715.65 (7218.68). Using the Mann Whitney test showed that there were differences in the mean sFlt-1 levels between subjects with preeclampsia and subjects without preeclampsia (p = 0.021).

Table 7: Relationship of age, parity, BMI, diastolic notches, uterine artery PI, levels of PlGF, sEng, sFlt-1 to early-onset preeclampsia

	Preeclampsia		P-value
	+(n =5)	- (n=65)	
Age, n (%)			
>35 y old	0	8 (100)	1,000a
≤35 y old	5 (8,1)	57 (91,9)	
Parity			
Primigravida	3 (9,6)	28 (90,4)	
Secundi Gravida	0	23 (100)	0,251a
Multigravida	2 (12,5)	14 (87,5)	
BMI, n (%)			
Overweight and Obese	1 (3,1)	31 (96,9)	0,169a
Underweight andNormoweight	4 (14,8)	23 (85,2)	
Diastolic Notches			
Yes	5 (14,3)	30 (85,7)	0,054a
None	0	35 (100)	
PIUterine A, mean(SD)	1,44 (0,30)	1,11 (0,35)	0,045b
PlGF, mean(SD)	411 (301,67)	866,77 (404,73)	0,016c
sEng, mean(SD)	8 (4,11)	5,17 (1,62)	0,113c
sFlt-1, mean(SD)	17943 (8346,99)	9715,65 (7218,68)	0,021c

[a]Fischer's Exact, [b]T Independent, [c]Mann Whitney

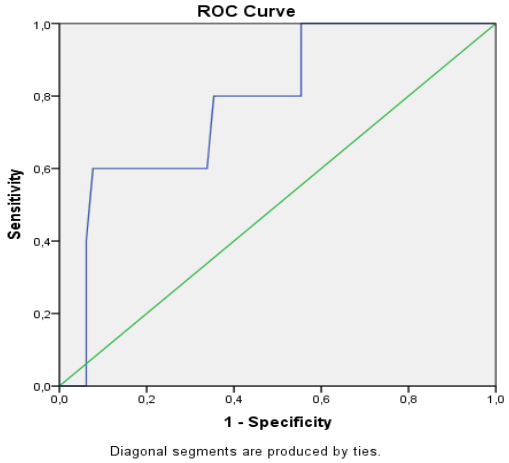

Fig. 5: ROC curve of uterine artery PI against preeclampsia

Prognostic value of the uterine artery pulsatile index, PlGF, sEng, sFlt-1 in predicting early-onset preeclampsia

From the results of the analysis using the ROC curve obtained p-value = 0.037 which means that the uterine artery PI in this study has the ability to predict the incidence of early-onset preeclampsia with an Area Under Curve (AUC) value of 78.2% (95% IK 59.3%-97%).

Based on the sensitivity and specificity curve in fig. 4.6, the Cut Off value for uterine artery PI is 1.282. By using a cut-off point 1,228, the sensitivity value of uterine artery PI was 80%, and specificity was 64.6%.

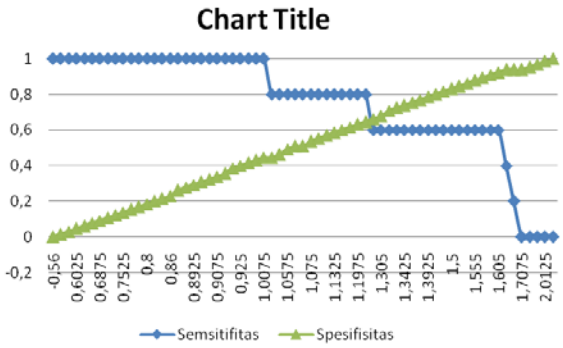

Fig. 6: The sensitivity and specificity curve of uterine artery PI to the incidence of early-onset preeclampsia

From the results of the analysis using the ROC curve obtained p = 0.016, which means that the PlGF in this study has the ability to predict the incidence of early-onset preeclampsia with an Area Under Curve (AUC) value of 82.5% (95% IK 61.5%-100%).

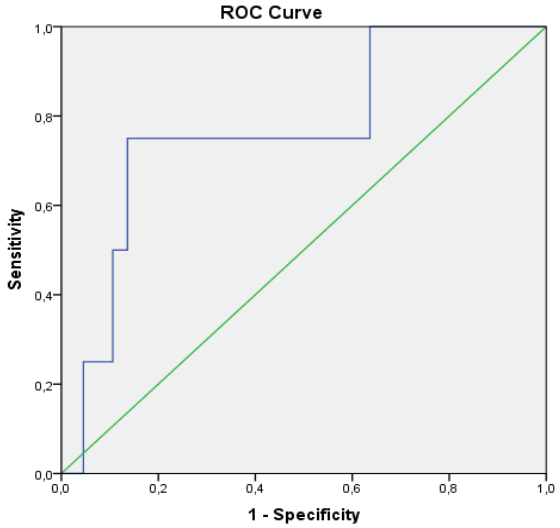

Fig. 7: ROC curves of PlGF against preeclampsia

Based on the sensitivity and specificity curves in fig. 8, the Cut Off value for PlGF levels was 441. Using the cutoff point 441, the PlGF sensitivity value was 80% and 87.7% specificity.

From the results of the analysis using the ROC curve obtained p = 0.113, which means that in this study did not have the ability to predict the incidence of early-onset preeclampsia.

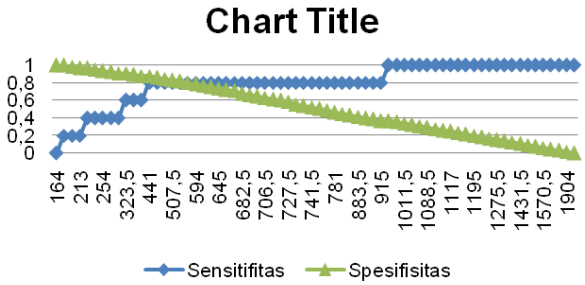

Fig. 8: PlGF sensitivity and specificity curves for early-onset preeclampsia

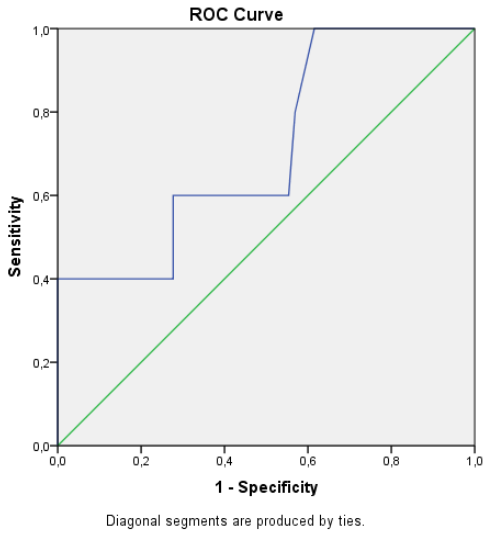

Fig. 9: ROC curves of the one against preeclampsia

From the results of the analysis using the ROC curve obtained p = 0.021 which means that sFlt-1 in this study has the ability to predict the incidence of early-onset preeclampsia with an Area Under Curve (AUC) value of 81.2% (95% IK 63.6%-98.9%).

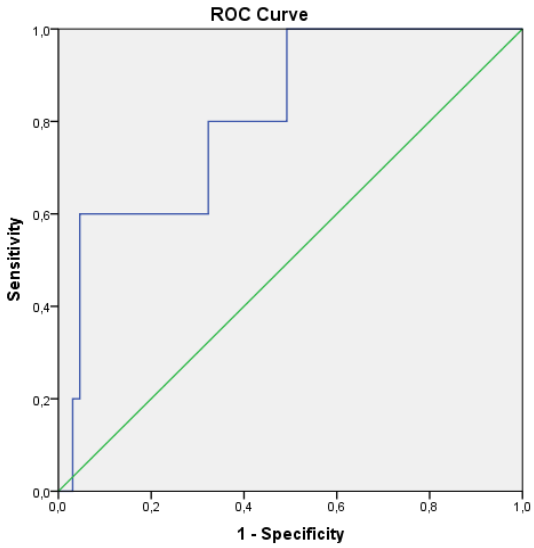

Fig. 10: ROC curves of sFlt-1 against preeclampsia

Based on the sensitivity and specificity curves in fig. 11, the Cut Off value for the sFlt-1 level is 10087.5. By using a cut-off point of 10087.5, the sensitivity value of sFlt-1 is 80% and the specificity is 67.7%.

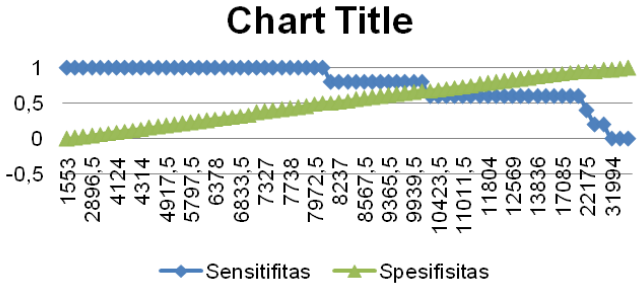

Fig. 11: The sFlt-1 sensitivity and specificity curve for the onset of early-onset preeclampsia

Table 8: Area under the curve for PlGF, sEng, sFlt-1 for early-onset preeclampsia

	Area under curve	P value	95% IK	
			Upper limit	Lower limit
PI Uterine Artery	78,2%	0,037	59,3%	97%
PlGF	82,5%	0,016	61,5%	100%
sEng	71,4%	0,113	47,7%	95%
sFlt-1	81,2%	0,021	63,6%	98,9%

Table 9: Sensitivity, specificity, the positive and negative predictive value of uterine artery PI, PlGF and sFlt-1 against early-onset preeclampsia

		Preeclampsia		Sensitivity	Specificity	NPP	NPN
		+	-				
IP. A. Uterina	≥ 1,228	4	23	80%	64,6%	14.,8	97,7%
	<1,228	1	42				
PlGF	≤441	4	8	80%	87,7%	33,3%	98,3%
	>441	1	57				
sFlt-1	≥10087,5	4	21	80%	67,7%	16%	97,8%
	<10087,5	1	44				
PlGF+sFlt-1				80%	98,50%	80%	98,50%
PlGF+Uterine Artery PI				40%	90,77%	25%	95,16%
sFlt-1+Uterine Artery PI				40%	96%	50%	95%

Multivariate analysis

The independent variables included in the multivariate analysis were the variables in the bivariate analysis with a p value<0.25, namely BMI, uterine artery diastolic notches, uterine arterial PI, PlGF, sEng, and sFlt-1. Multivariate analysis in this study used a multiple logistic regression test with the enter method.

From the results of multiple logistic regression analysis, it can be seen that only one independent variable can predict the incidence of early-onset preeclampsia significantly, namely PlGF with Exp (B) = 28.5 (Odds Ratio) (95% IK 2,821-287,948) which means that the subject with PlGF levels<441 would be at risk of experiencing early-onset preeclampsia by 28.5 times compared to subjects with PlGF levels ≥441.

The resulting value is 0.017, meaning that the subject's chances of experiencing early-onset preeclampsia is 1.7%. In the same way, when entering subject number 5 with a PlGF level of 253 (given code 1), the equation obtained is calculated with a value of 0.333, meaning that the subject's chances of experiencing early-onset preeclampsia is 33.3%.

Table 10: Multivariate analysis results of variables that can predict the incidence of early-onset preeclampsia

	Coef	P-value	Esp (H)	95% IK Exp (B)	
				Lower	Upper
Selection 1					
BMI	1,377	0,459	3,963	0,104	151,374
Diastolic Notches	-17,378	0,998	0,000	0,000	.
PI uterine A.	-1,110	0,584	0,329	0,006	17,540
PlGF	1,428	0,345	4,169	0,215	80,926
sEng	0,272	0,295	1,313	0,789	2,184
sFlt-1	-2,287	0,124	0,102	0,006	1,869
Konstanta	-3,656	0,196	0,026		
Selection 2					
BMI	1,982	0,267	7,258	0,220	239,481
PI uterine A	-2,102	0,258	0,122	0,003	4,668
PlGF	1,705	0,267	5,499	0,271	111,493
sEng	0,344	0,175	1,411	0,858	2,318
sFlt-1	-2,092	0,170	0,123	0,006	2,443
Konstanta	-4,831	0,070	0,008		
Selection 3					
PI uterine A	-2,331	0,182	0,097	0,003	2,977
PlGF	2,064	0,156	7,874	0,456	135,845
sEng	0,315	0,229	1,370	0,821	2,288
sFlt-1	-2,196	0,124	0,111	0,007	1,826
Konstanta	-3,327	0,077	0,036		
Selection 4					
PI uterine A	-1,639	0,257	0,194	0,011	3,308
PlGF	2,866	0,039	17,573	1,153	267,842
sFlt-1	-2,592	0,054	0,075	0,005	1,050
Konstanta	-1,902	0,172	0,149		
Selection 5					
PlGF	3,628	0,005	37,632	3,002	471,759
sFlt-1	-2,491	0,058	0,083	0,006	1,091
Konstanta	-3,104	0,003	0,045		
Selection 6					
PlGF	3,350	0,005	28,500	2,821	287,948
Konstanta	-4,043	0,000	0,018		

DISCUSSION

PIGF

In the overall sample, it was known that the mean PlGF level was 834.21 pg/ml (SD = 413.75) (table 4). In the sample of preeclampsia in this study the mean PlGF was 411 pg/ml at 22-24 w gestation (table 7). In preeclampsia, serum PlGF levels are found to decrease, but there is no standard reference level for PlGF levels, from various studies a cut-off point value as a reference material for subsequent research has been conducted. Different studies found that the mean PlGF levels in preeclampsia are the following 125 pg/ml at 20-37 w' gestation [7]; 95 pg/ml in midtrimester gestational age [8]; 38.7 pg/ml at 32-37 w' gestation [9]; 54 pg/ml at 20-34 w' gestation [10]; 83.75 pg/ml at 20-30 w gestation [11]; 239.75 pg/ml at 25-40 w' gestation [12]. In this study PLGF levels tend to be high among several other studies, this can be due to the lack of sample size in the preeclampsia data.

Along with the increasing number of studies in the field of PE biormarkers, recent studies have focused on various peptides that mediate the process of angiogenesis. Angiogenesis is a process of blood vessel growth that is very important in the normal growth of the placenta. There are 2 types of peptides that chill the process of angiogenesis, namely Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF). The imbalance between the two peptide angiogenesis is believed to play a major role in the occurrence of PE in pregnant women [13].

In pregnant women who suffer from PE, VEGF levels are lower than pregnant women who do not suffer from PE, as well as PlGF, in pregnant women with PE obtained lower levels compared to pregnant women without PE. In several studies of cancer patients who received anti-VEGF showed symptoms of hypertension and proteinuria, this showed that anti-VEGF could play a role in the appearance of PE symptoms in pregnant women [14].

PlGF can be detected easily through urine examination, while VEGF requires ELISA examination in order to be detected accurately, therefore the use of the ratio of PlGF and sFlt-1 during middle pregnancy can be a predictor of PE [15].

sEng

The mean level of all samples was 5.37 pg/ml (SD = 1.99) (table 4.4). The mean age in preeclampsia samples was 8.0 ng/ml at 22-24 w gestation (table 4.7). In other studies, the mean levels of sEng in preeclampsia were 53.3 ng/ml at less than 34 w' gestation (Rios, *et al.*, 2016); 11.08 ng/ml at 19-24 w gestation [16]; 60.9 ng/ml in the first and second trimester of gestational age [17]; 69.2 ng/ml in the age of the third trimester of pregnancy [18]; 11.58 ng/ml at gestational age less than 37 w. [19] In several studies compared to the results of this study, various levels of sEng were found that also did not yet have a standard level that became a reference in the examination of preeclampsia.

Soluble endoglin or abbreviated as the task of binding TGFB1 and TGFB2. It is an antiangiogenic factor that plays a role in inhibiting TGFB1 binding to its receptors, resulting in a disruption in the production of nitric oxide (NO), vasodilation, and capillary formation by endothelial cells *in vitro* [20].

In normal pregnancy, blood levels decrease between the first and second trimesters, and in patients with PE, blood levels remain high and tend to continue to increase. sEng Also associated with the severity of the disease and its level decreases after the baby and placenta are born to pregnant women with PE. May be used as one of the biomarkers that can predict the appearance of PE in pregnant women, this is because it can increase several weeks before the appearance of PE symptoms in pregnant women. Furthermore, in pregnant women who suffer from preterm PE, there will be an increase in blood levels up to two times at 17-20 w of pregnancy [21].

sFlt-1

The entire sample studied both normal pregnancy and preeclampsia was known to have an average sFlt-1 level of 10303.31 pg/ml (SD = 7544.99) (table 4). And sFlt-1 measurement results in cases of preeclampsia alone showed an average of 17943 pg/ml at 22-24 w gestation (table 7). In preeclampsia, sFlt-1 serum levels will be increased. Various studies that can be used as a reference in preeclampsia are with sFlt-1 levels as follows 1048 pg/ml at the age of the first trimester of pregnancy [22]; 37700 pg/ml at gestational age less than 32 w [23]; 310.22 pg/ml at the age of the second trimester of pregnancy and 514.23 pg/ml at the age of the third trimester of pregnancy [24]; 7328-20414 pg/ml at the age of the second trimester of pregnancy [25]. In another study found something similar to the results of this study, which levels of sFlt-1 tended to increase.

Soluble Flt-1 (sFlt-1) is an antiangiogenic biomarker. sFlt-1 circulates freely in the serum by binding to and neutralizing VEGF and PlGF. Several studies have shown an association between increased sFlt-1 and PlGF. SFlt-1 levels begin to increase from 5 w before the onset of PE and the levels remain high until the onset of preeclampsia persists during the pregnancy process (Levine et al., 2006). The sFlt-1 level is directly correlated with the severity of the disease and inversely proportional to the time of onset of proteinuria and hypertension. A method of PE detection must be able to distinguish PE from hypertensive disorders in other pregnancies (gestational hypertension and chronic hypertension) [26].

The clinical use of serum concentrations of antiangiogenic proteins in distinguishing hypertensive disorders in pregnancy has been evaluated, the sensitivity and diagnostic specificity of sFlt-1 for the differentiation of PE from gestational hypertension and chronic hypertension are 84% and 95%. Based on this, sFlt-1 is an effective and accurate biomarker candidate to be used as a diagnostic tool because it can be used to differentiate PE from gestational hypertension and chronic hypertension [27].

Measurement of sFlt-1 in plasma showed a sensitivity of 89% and a specificity of 90% in early onset PE (<34 w) compared to a percentage in the final phase of PE (>34 w) with a sensitivity of 55% and a specificity of 58%. Urine screening by performing a PlGF assay, followed by blood confirmation by checking the sFlt-1/PlGF ratio is a promising strategy [28].

The mechanism of circulating sFlt-1 and PlGF in the blood which is used as a parameter to examine the ratio of both is as follows, it is known that sFlt-1 is a causative factor or cause of PE. sFlt-1 acts as an antagonist of VEGF and PlGF by binding to these molecules and decreasing the level of VEGF and PlGF circulating in the blood. The decrease in PlGF and VEGF results in changes in vasodilation which cause hypertension [29].

From this mechanism, it is known that the concentration of sFlt-1 circulating in the blood of pregnant women with PE will be found to be increased, while the free PlGF concentration has decreased in the blood. The sFlt-1/PlGF ratio has been proposed as an index of antiangiogenic activity that reflects changes in both biomarkers and is also a better way to diagnose PE compared to just one measure. Regarding the efficiency of serum biomarkers, a study has been carried out using the sFlt-1/PlGF ratio clinical tool in stratifying patients at risk of PE possibly reducing cost (effective cost) and resources [30].

Cost reduction is needed in the detection using the sFlt-1/PlGF method due to the high sensitivity and specificity of the method so that no additional testing methods are needed to confirm the diagnosis of PE disease [31].

Several clinical studies have recommended the sFlt-1/PlGF method in the diagnosis of PE. Based on research conducted by Zeisler, the ratio of sFlt-1/PlGF can be used to predict and detect the possibility of clinical PE events [32].

The same was stated by Klein *et al.*, The use of sFlt-1/PlGF as a biomarker is very potential to be developed in clinical practice so that it can help therapeutic management and hospitalization of patients with symptoms of PE, eclampsia, hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome [33].

Relationship of diastolic notches, uterine artery pulsatile index, PlGF, sEng, sFlt-1 to the incidence of early onset preeclampsia

Of the 35 samples of pregnant women who had diastolic notches evidence, there were 5 samples that had early onset preeclampsia, with a p value of 0.054 (>0.05) that did not have a significant

difference in relationship (table 7), this was probably due to the number of samples that had preeclampsia didn't enough. Several other studies that also examined diastolic notches events on preeclampsia, namely Kushtagi and Emani, 2016 examined the presence or absence of diastolic notches pregnancy in patients with hypertension at 11-14 w of gestation in India, with a p value of 0.612 which means there is no significant difference between patients normal and patients with hypertension; [34] Doherty, *et al.*, 2014 also examined this but included pregnant patients with fetal growth retarded both unilateral and bilateral diastolic notches values of p value 0.7 (not significantly different) [10].

But there are other studies also found that there are significant differences between patients with normal pregnancies and patients with pregnancies that have preeclampsia, namely the Vartun, Flo, Widnes, and Acharya, 2016 study with p values <0,0001 (p<0.05) is very significant with the finding of 23 patients with diastolic notches preeclampsia compared with 4 patients with diastolic notch also but did not experience preeclampsia [35].

Average PI uterine artery in subjects with early onset preeclampsia was seen to be higher with a mean of 1.44 (SD = 0.30) compared to subjects without preeclampsia with a mean of 1.11 (0.35) with a p value of 0.045 which means it has a significant value to distinguish cases preeclampsia (table 7). From previous research studies also dominated by significant results that are in line with this study, which makes the initial step to do non-invasive screening. Previous research was in the form Kushtagi and Emani, 2016 with a p value of 0.013 but was carried out on samples with gestational hypertension, despite other studies by Vartun, Flo, Widnes, and Acharya,, Yu, Cui, Chen, and Chang and Narang, Agarwal, Das, Pandey, Agrawal, and Aliwith a very significant p value<0,0001 performed in pregnant women in the early trimester that ends in preeclampsia [34-37].

Specific studies according to Konishi and Katabuchi, 2018 focus on soluble antiangiogenic factors such as fms-like tyrosine kinase-1 (sFlt-1) which is a receptor of soluble endothelial vascular-1 growth factor (VEGFR-1) and pro placental growth factor-angiogenic (PlGF). PlGF itself in this study found significance between the relationship of PlGF to the incidence of early onset preeclampsia with a p value of 0.016 (p<0.05) (table 7) [38]. Low circulating PlGF may be a consequence of an abnormal initial occurrence in placentation and factors that contribute to abnormal growth that continues during the last half of pregnancy.

This is what causes PlGF levels to decrease in cases of preeclampsia. In several other studies, many support that a decrease in PlGF levels is a predictor of the incidence of preeclampsia that has a significant relationship, as according to Masuyama, Nakatsukasa, Takamoto, and Hiramatsu, 2007 with an average PlGF value of preeclampsia patients 137.6 pg/ml compared to the average value the average control patient's PlGF was 248.6 pg/ml (p value<0.01) [17].

Al-jamcil who conducted the most recent study on early onset preeclampsia and late onset preeclampsia with the same results as a decrease in PlGF levels but with higher levels in early onset preeclampsia (159.81 pg/ml) significant p value 0.002 compared to late onset (slow onset (PlGF) 109.91 pg/ml) significant p value 0.008 [39]. Previous studies have also been conducted by Bian, Shixia, and Duan, which showed a significant difference in the reduction in PlGF levels in preeclampsia performed in pregnant women in the first trimester of pregnancy with an average of 115.72 pg/ml compared to controls 217.30 pg/ml (p value<0,001); [40] and by Gannoun, *et al.*, in the following year who conducted more in-depth studies dividing based on gestational age intervals with all the results of research on PlGF levels found to be significant that at 24-29 w gestation the average PlGF level was 58.62 pg/ml ending in early onset preeclampsia (p value 0.007) [41].

From the results of this study, a p value of 0.113 was not found to be significantly different between sEng levels in early onset preeclampsia and the mean value in preeclampsia 8 ng/ml which was slightly higher than the mean in controls which was 5.17 ng/ml (table 4.7). In various studies there are still various levels of ED that have not been used as a standard, such as according to EL-Said, Mohammed, EL-Ashmawi, and Saad, 2013 the mean sEng levels in preeclampsia also increased (11.06 ng/ml) compared to the control mean (5, 92 ng/ml) but with a p value<0.01, which means that significant differences were found, similar to the Perucci research, 2014 which results in an increase in sEng levels in preeclampsia both early onset and later onset with a p value of 0.001 [16]. As well as Masuyama, Nakatsukasa, Takamoto, and Hiramatsu, 2007 research which had very different levels of sEng in preeclampsia that is 60.9 ng/ml with a control of 11.2 ng/ml (p value<0.01) [17].

Even though from the various studies above there were significant results, but there are other studies which were also not significantly different as in this study, according to K. E. Duhig, 2015 with a p value of 0.058 (p value>0.05) which was conducted in one woman's examination. second trimester pregnancy was found to have a mean level of 2.78 ng/ml which was lower than the control of 3.52 ng/ml but in other pregnant women samples at the age of the third trimester which was also carried out in this study found significant results (p value 0.001) [42].

The results of other studies showed the mean level of soluble endoglin (sEng) of maternal serum with early onset preeclampsia (PEAD) was 41.47±13.88 ng/ml whereas it was lower in slow onset preeclampsia (PEAL) 33.19±15.99 ng/ml [43].

The mean sFlt-1 level in preeclampsia from the results of this study was 17943 pg/ml which was far higher than normal pregnancy without preeclampsia which was 9715.65 pg/ml with a significant significant difference (p value 0.021) (table 7). Based on the theory it is also known that sFlt-1 levels tend to be reminiscent of patients who will experience preeclampsia and who already have preeclampsia, because sFlt-1 itself is also a more specific VEGF mediator in the event of inflammation. Some other studies that also support the results of this study are Magee LA, who found an increase in sFlt-1 levels compared to controls in first trimester pregnant women with a p value of 0.001, [44] also Hassan, Rund, and Salama, 2013 with a very significant p value of<0,0001.[8] A similar study was conducted by Andersen, *et al.*, 2015 who compared the sampling and examination using Elecsys and KRYPTOR which is a type of ELISA with also significant results namely Elecsys (p value<0,0001) and KRYPTOR (p value<0.001) [11].

By Shu, who examined pregnant women with early onset preeclampsia and late onset preeclampsia also produced the same significant value of differences in sFlt-1 levels compared with pregnant women who did not experience preeclampsia, with successive p values of 0.001 and 0.002 [45].

sFlt-1 is a type of membrane binding Flt-1. sFlt-1 circulates freely in the serum, and is responsible for binding and neutralizing VEGF and PlGF. Several studies have found that there is a relationship between increasing sFlt-1 and PE [21]. The sFlt-1 level begins to increase at 5 w before the onset of PE and the levels still increase compared to women who do not suffer from PE. sFlt-1 is believed to be closely related to the severity of the disease. The level of sFlt-1 in pregnant women with PE will decrease if the baby and placenta have been born. In addition, nulliparous mothers obtained higher levels of sFlt-1 compared to multiparous mothers [15].

Maynard *et al.* Said that "mRNA from sFlt-1 is made by the placenta of pregnant women suffering from PE". Furthermore, Maynard found that pregnant mice receiving sFlt-1 adenovirus injections suffer from hypertension and proteinuria, as well as glomerular endotheliolysis, and several pathological symptoms such as PE [46].

In a study conducted by Staff *et al.*, it was suggested that sFlt-1 was produced mostly from the placenta. In mothers with PE, sFlt-1 levels were increased 29 times compared to fetal sFLT-1 levels, this indicates that there was no role of the fetus in increasing sFlt-1 levels in pregnant women suffering from PE. Furthermore, there was a significant increase in sFlt-1 levels in mothers with PE impending eclampsia and in patients with systemic lupus erythomatosus [32].

Predictors of early onset preeclampsia

Other studies have also conducted multivariate statistics to look for cut values to help clinicians diagnose preeclampsia, with various cutoff values. According to Hassan, Rund, and Salama, 2013 [8], the

cutoff point values were respectively sFlt-1 3198 pg/ml and PlGF 138 pg/ml with AUC values of 0.875 and 0.855. Also by N Ogorman, *et al.*, 2017 which is a recent study with cutoff points sFlt-1 3369 (AUC 0.89) and PlGF 60.4 (AUC 0.94) [47]. Chen in 2009 had conducted an analysis of various previous studies to record the cut-off point value. Although this research was found to be insignificant, a cut-off point was not searched for, the research collected by Chen was Levine, 2006 gestational age 13-20 w 7.9 ng/ml, 21-32 w 7.2 ng/ml, 33-42 w 13.6 ng/ml; Salahuddin, 2007 24.8 ng/ml [18]; Bauman, 2008 5 ng/ml; and Stepan 2008 4.14 ng/ml [48, 51].

In multivariate analysis with six levels of selection, to determine the ability of variables to predict early onset preeclampsia it is known that the single best examination that can be performed as a predictor is serum PlGF levels with a p value of 0.001 (very significant). In the previous selection combined with other variables namely sFlt-1 and ultrasound examination and anamnesis from the patient's characteristics, it was found that the p value was also<0.05 which was significant for the examination, although it turned out that only one examination was sufficient as a predictor of early onset preeclampsia. The results of this study are very useful as well as generating effective cost in financing in diagnosing preeclampsia itself, and carried out at 22-24 w of gestation.

Evaluation of various angiogenic and antiangiogenic factors in maternal serum and plasma has been tested as a diagnostic marker of preeclampsia for possible use in predicting the development of preeclampsia and serum levels of sFlt-1 were found to be involved in preeclampsia [46].

Based on studies conducted in experimental animals to see the pathological role of antiangiogenic factors sFlt-1, the pregnant mice showed preeclampsia syndrome after adenoviruses that expressed sFlt-1 [46, 49]. In humans, serum levels of placental growth factor (PlGF) are reduced in women with preeclampsia [50]. Increased sFlt-1 and decreased PlGF have appeared in maternal serum 5 to 10 w before the onset of preeclampsia and it has been determined that these changes in sFlt-1 and PlGF contribute to the pathogenesis of preeclampsia [51].

The potential prediction of sFlt-1 and sEng has attracted great attention in the field of research in preeclampsia in recent years, the latest findings provide evidence showing that sFlt-1 and sEng levels are related to the pathology of preeclampsia [52].

The results of this study are consistent with research conducted by Lee *et al.* in 2007 which found that serum sFlt-1 concentrations were significantly increased in patients with preeclampsia compared to uncomplicated pregnant women. There is a positive correlation between serum concentrations of sFlt-1 with systolic and diastolic blood pressure, and concludes that sFlt-1 is associated with the pathogenesis of preeclampsia [53].

Likewise with the results of a study conducted by Reddy *et al.* in 2009 which found that sFlt-1 and sEng levels were significantly found to be higher in patients with preeclampsia compared to normal pregnant women. In labor, sFlt-1 levels increase significantly when maximal dilatation in patients with preeclampsia, before decreasing in the first 24 h post partum [54].

Noori *et al.* in 2010 found that pregnant women with preeclampsia had higher levels of sFlt-1 compared to normotensive pregnant women. During pregnancy, inhibition of vascular endothelial growth factor by sFlt-1 causes disruption of endothelial growth and proteinuria [55].

Govender *et al.* in 2012 found serum sFlt-1 concentrations in the normal blood pressure group (9,603±1,797 pg/ml) were significantly smaller than those in the early onset preeclampsia group (26,682±5,482 pg/ml) (p<0.05) and concentrations Serum sFlt-1 in the early onset and late onset preeclampsia group (16,069±4,305 pg/ml) was higher than the chronic hypertension group (8,811±2,008 pg/ml), but this difference was found to be not significantly different. Two things that become a limitation in this study are the small sample size that can affect the results statistically, and do not measure/assess serum angiotensin II levels and angiotensin I autoantibodies [56].

Antiangiogenic protein sFlt-1 which inhibits placental proangiogenic protein (growth factor PlGF and VEGF) is reported to increase before the onset of preeclampsia. Circulation of sFlt-1 correlates with the onset of hypertension or proteinuria as a sign of preeclampsia. Imbalances in circulating angiogenic factors are associated with vascular endothelial dysfunction and maternal syndromes with preeclampsia [51].

The presence of dynamic VEGF and PlGF proangiogenic factors in pregnancy requires endothelial cells to function properly and be able to survive for a long time when sFlt-1 plasma levels rise, they can reduce circulating VEGF and PlGF levels below the critical threshold needed for maintenance of blood vessels in adults. The resulting endothelial dysfunction can disrupt the blood-brain barrier and cause intracranial hypertension, cause edema in the liver, and affect glomerular function [51].

Eremina *et al.* in 2003 showed that glomerular capillary function was under VEGF control. That is, when the VEGF level in the kidney prodosit drops 50%, glomerular endothelial cells swell, capillary loops collapse, and proteinuria develops as occurs in patients with preeclampsia [57].

Endothelial dysfunction can also interfere with homeostasis and trigger thrombocytopenia. By inducing vasodilation, VEGF also induces hypotension, and thus circulation of low levels of VEGF will cause high blood pressure, another feature of preeclampsia. Thus, the sFlt-1 hypothesis allows a proposed unifying model, explaining some of the symptoms of preeclampsia. During pregnancy, inhibition of vascular endothelial growth factor with sFlt-1 results in impaired endothelial function and proteinuria [58].

In line with Rahmi's 2016 study found that women with preeclampsia had a greater increase in sFlt-1 levels compared to women with gestational hypertension or normotensive pregnancy. However, towards the end of pregnancy, there was an increase in sFlt-1 levels in all groups, including pregnant women with normal blood pressure, which could contribute to the increase in albuminuria seen in healthy pregnancies [59].

Rahmi's research in 2016 showed that the mean serum sFlt-1 level in the severe early-onset/eclampsia group was higher (4.69±0.96 ng/ml) compared to the group of severe late-onset/eclampsia (2.39±0), 57 ng/ml), and also from the normal pregnancy group (1.23±0.42 ng/ml), and there were very significant differences between the three study groups. Higher serum sFlt-1 levels found in the early onset and late onset preeclampsia group compared to normal pregnancy indicate that there is an imbalance of angiogenic and anti-angiogenic factors that begin at the level of the placenta at the beginning and end of the onset of preeclampsia [59].

CONCLUSION

The value of pulsatile uterine artery index with a cut-off point 1,228, Area Under Curve (AUC) of 78.2% (95% IK 59.3%-97%), sensitivity 80%, specificity 64.6%. PlGF levels with 441 pg/ml cut-off point, Area Under Curve (AUC) of 82.5% (95% IK 61.5%-100%), 80% sensitivity, specificity 87.7%. Levels of sFlt-1 with a cut-off point of 10087.5 pg/ml, Area Under Curve (AUC) of 81.2% (95% IK 63.6%-98.9%), sensitivity 80%, specificity 67.7 %. From the results of bivariate analysis, it is known that the combined examination of PlGF and sFlt-1 has a sensitivity of 80% and specificity of 98.50%, examination of PlGF and uterine artery PI has a sensitivity of 40% and specificity of 90.77%, and examination of sFlt-1 and uterine artery IP has a sensitivity of 40% and a specificity of 96%. From the results of multivariate analysis of the 6th level of selection, it was concluded that examination of PlGF levels alone was sufficient as a predictor of early onset preeclampsia.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest in this research

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