



# Determination of Urine Protein Levels and Analysis of Differences in Vascular Endothelial Growth Factor Levels between Early Onset and Late Onset Preeclampsia

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## Abstract

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**BACKGROUND:** Preeclampsia is a disease with the high mortality rate. Data indicate that 23.5% of maternal deaths due to preeclampsia. Urine protein testing is essential for pregnant women to discover the function of kidney during pregnancy and to identify the presence of preeclampsia, both mild and severe, which it can lead to eclampsia. Preeclampsia based on onset can also be detected by examining serum biomarkers of vascular endothelial growth factor (VEGF).

**AIM:** The aim of this study was to overview the urine protein levels and analyzed the differences serum VEGF levels between the early and late onset of preeclampsia.

**MATERIALS AND METHODS:** This study used design of the cross-sectional comparative study in RSUP DR. M Djamil Padang, Pariaman Hospital, Aisiyah Pariaman Hospital, and Padang Pariaman Regional Hospital for 2 years. The population in this study consisted of patients with early and late onset preeclampsia who went to the study site during that time and met the inclusion criteria. Sampling with consecutive sampling consisted of 28 people per group.

**RESULTS:** The results revealed that the number of patients who had urine protein levels with a value of 2+ at the early onset was 14 and at the late onset was 12, meanwhile for patients with a urine protein content value of 3+ at the early onset it was 14 and at the late onset was 10. For the category urine protein with a value of 4+ amounted to six people only in the late onset group. The results of the normality test showed that the mean value of VEGF levels at early onset and late onset was 3.91 pg/ml and 4.3 pg/ml, respectively. Thus, the level of VEGF serum had an abnormal distribution and it led to the t-test data could not be tested. So then, the testing was done using a non-parametric test, namely, the Mann-Whitney test. The mean levels of VEGF, respectively, based on the Mann-Whitney test for early onset preeclampsia and late onset preeclampsia were 29.75 ng/mL and 30.25 ng/mL. VEGF in early onset preeclampsia is lower than late onset preeclampsia.

**CONCLUSION:** Based on the results obtained, it can be concluded that there was no significant difference in the level of VEGF in early onset preeclampsia and late onset preeclampsia ( $p = 0.42$ ).

## Introduction

Preeclampsia is a problem in the field of obstetric health, due to this disease is a high cause of death, namely, 50,000–70,000 female deaths and 500,000 infant deaths each year [1]. The maternal mortality rate (MMR) in Indonesia due to preeclampsia together with infection and bleeding occupies the highest place in ASEAN [2]. Indonesia is one of the developing countries with the high MMR and perinatal mortality with the second rank in the Southeast Asia region and the 3<sup>rd</sup> rank in ASEAN [3].

Dealing with the onset of occurrence, preeclampsia can be divided into two parts, namely, early onset preeclampsia and late onset preeclampsia [4]. Early onset preeclampsia is preeclampsia that occurs at

gestational age <34 weeks and late onset preeclampsia is preeclampsia that happens at >34 weeks of gestation [5]. Early onset preeclampsia is usually more dangerous than late onset preeclampsia [6]. Early onset preeclampsia is the type that causes the highest maternal and fetal morbidity and mortality rates. In the early onset of preeclampsia, the spiral artery formation is not perfect, resulting in hypoperfusion of the placenta so that the supply of nutrients to the fetus is reduced and eventually fetal growth restriction (FGR) occurs [7]. Whereas, in the late onset of preeclampsia merely experience a slight decrease in the diameter of the spiral arteries and there are no signs of FGR [8].

The clinical appearance of early onset and late onset preeclampsia is similar but there are differences in prognosis, complications, and outcomes of mothers and babies [9], [10]. Early onset

preeclampsia is usually more dangerous than the late onset preeclampsia [11]. Early onset preeclampsia is the type that causes the highest maternal and fetal morbidity and mortality rates. In the early onset of preeclampsia, the form of spiral artery is not perfect, so then it can lead to the hypoperfusion of the placenta and the supply of nutrients to the fetus is reduced and eventually FGR occurs. Whereas, it is also found that there is merely slight decrease of the diameter of spiral arteries and there are no signs of FGR in the late onset of preeclampsia [12].

Various attempts have been undertaken to reduce maternal mortality, one of which is by conducting the early detection of preeclampsia, among others by examining factors during pregnancy, but these efforts are currently still limited. Several studies have recommended vascular endothelial growth factor (VEGF) testing to detect the possibility of preeclampsia [13].

The examination of urine protein examination is also carried out to pregnant women, this is one type of laboratory examination to discover the function of kidney during pregnancy and identify the presence of both mild and severe preeclampsia which can lead to eclampsia [14]. Proteinuria detection is very essential in the diagnosis and handling the hypertension in pregnancy. Proteinuria is the last symptom to appear in preeclamptic patients. However, eclampsia can occur without proteinuria. Proteinuria in preeclampsia is an indicator of danger to the fetus, low birth weight, and an increased risk of perinatal death [15]. In this study, a study was conducted regarding the description of urine protein levels and analysis of VEGF levels in patients with early onset preeclampsia and late onset preeclampsia.

## Methods

### Research population

The population of study was patients with early onset and late onset preeclampsia who went to RSUP DR. M Djamil Padang, Pariaman Regional Public Hospital, Aisiyah Pariaman Hospital, and Padang Pariaman Regional Public Hospital during the study period. The study sample was pregnant women with preeclampsia who met the inclusion and exclusion criteria and obtained 28 samples of each for early onset preeclampsia and late onset preeclampsia.

The inclusion criteria in this study were patients that diagnosed with preeclampsia by experts based on clinical appearance, obstetric examinations, and supporting examinations and were willing to be involved in the study. The exclusion criteria in this study were patients with chronic hypertension, patients with

gestational hypertension, patients with malignancy, and patients with severe infections.

### Sample of blood

The blood sample taken was venous blood using a syringe aspiration procedure. 4 ml of blood was taken and put into a blood chemistry vacutainer. The vacutainer that already contained of the sample, then, it was given a label name, medical record number, place of birth date, and the name of the patient's birth mother. This sample was put in a vacutainer transport box for later examination in the laboratory.

### Statistical analysis

Statistical analysis of this study used the Statistical Package for the Social Sciences Software (SPSS), version 21.0 (SPSS Inc., Chicago, IL) normality test using the Saphiro–Wilk test. VEGF  $p = 0.00$  ( $p > 0.05$ ). This data showed that the data that owned were not normally distributed. The results of the data distribution that were not normal and it caused the t-test data testing could not be undertaken, so that data testing was carried out using a non-parametric test, namely, the Mann–Whitney.

## Results

### Patient characteristics

The study sample was pregnant women with preeclampsia who met the inclusion and exclusion criteria and this study obtained 28 samples of each for early onset preeclampsia and late onset preeclampsia. The data of patient characteristics are shown in Table 1.

**Table 1: Patient characteristics data**

Parameters	Early onset Preeclampsia n = 28 (Mean ± SD)	Late Onset Preeclampsia n = 28 (Mean ± SD)
Age (y)	32.92 ± 6.06	32.57 ± 6.61
Body Weight (kg)	77.32 ± 5.26	82.99 ± 5.40
Height (cm)	157.9 ± 4.09	157.75 ± 3.08
Gestational age (week)	30.85 ± 1.70	38.53 ± 1.08
Systolic Pressure (mmHg)	160.85 ± 13.93	166 ± 10.3
Diastolic Pressure (mmHg)	106.85 ± 5.59	107.64 ± 6.32
Urine Protein Level	2.5 ± 0.5	2.78 ± 0.77

### Determination urine protein level

Examination of urine protein that can be undertaken on pregnant women is one type of laboratory examination to discover the function of kidney during pregnancy and to identify the presence of preeclampsia, both mild and severe, which can lead to eclampsia. The data from the urine protein examination are shown in Figure 1.

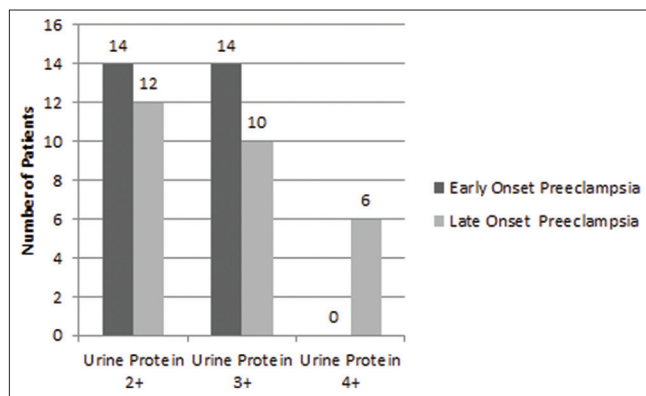


Figure 1: Overview of urine protein levels

### The examination result of VEGF serum

Preeclampsia begins in the first trimester with an asymptomatic phase characterized by insufficiency in the trophoblast invasion process and incomplete remodeling of the spiral arteries. Both of these processes have a role in the increase of oxidative stress and the occurrence of systemic endothelial dysfunction which will lead to preeclamptic symptoms in the final phase of the disease [16]. The role of VEGF as a regulator of angiogenic processes in early pregnancy is quite high and the level of VEGF on the human placenta is inconsistent in the preeclampsia condition. So then, the VEGF has function to detect the possibility of preeclampsia. The result of measuring the serum VEGF levels is shown in Table 2.

Table 2: Data values for serum level of VEGF (pg/ml)

Patients number	Early onset preeclampsia (pg/ml)	Late onset preeclampsia (pg/ml)
1	8.47	0.204
2	4.502	1.031
3	1.692	1.857
4	3.51	14.422
5	0.535	5.824
6	1.031	3.676
7	2.188	5.66
8	3.345	4.668
9	3.015	6.486
10	3.676	4
11	0.039	6.652
12	1.692	5.494
13	3.51	4.998
14	3.841	1.031
15	2.519	0.865
16	4.668	3.18
17	4.833	3.51
18	6.817	4.006
19	0.039	5.494
20	2.023	2.188
21	6.652	1.196
22	10.454	5.494
23	1.527	4.502
24	8.635	3.345
25	7.809	4.502
26	9.131	6.817
27	0.369	6.652
28	2.849	3.015
Mean ± SD	3.9 ± 2.8	4.3 ± 2.6

In accordance with the normality test value, serum VEGF levels have an abnormal distribution. The extreme value that appears in sample number 4 is a sample of late onset preeclampsia with a value of 14,422 pg/ml. The results of the data distribution that is not normal, it can cause the t-test data testing cannot be undertaken, so that data testing was undertaken using a non-parametric test, namely, the

Mann–Whitney. The following are the results of the Mann–Whitney test.

### Discussion

Based on the data shown in Figure 1, data obtained that the number of patients who have urine protein levels with a value of 2+ at the early onset is 14 and at the late onset is 12, while for patients with a urine protein content value of 3+ in the early onset is 14 and at the late onset is 10. For the category of urine protein with a value of 4+, six people are only in the late onset group. Proteinuria detection is very important in the diagnosis and management of hypertension in pregnancy. Proteinuria is the last symptom to appear in preeclamptic patients. However, preeclampsia can also occur without proteinuria. Proteinuria in preeclampsia is an indicator of danger to the fetus, low-birth weight, and an increased risk of perinatal death.

Table 3 shows that there is no significant difference in VEGF levels in early onset preeclampsia and late onset preeclampsia (p = 0.42). The mean levels of VEGF, respectively, based on the Mann–Whitney test for early onset preeclampsia and late onset preeclampsia were 29.75 ng/mL and 30.25 ng/mL. VEGF in the early onset of preeclampsia is lower than the late onset of preeclampsia.

Table 3: Mann–Whitney test results of VEGF levels in early onset and late onset preeclampsia

Groups	Mean Rank	Sum Rank	p-value
Early Onset Preeclampsia	29.75	749	0.422
Late Onset Preeclampsia	30.25	749	0.422

Preeclampsia is closely related to antiangiogenic properties which have a role in the formation of the initial vascularization of the placenta [17]. It is in contrast to VEGF which is classified as a proangiogenic biomarker and is a glycoprotein involved in angiogenesis [18]. VEGF has function to increase proliferation, migration, survival in endothelial cells, and increase capillary permeability [19]. Angiogenesis that promoted by VEGF can occur in both physiological and pathological conditions. Synthesis of VEGF is stimulated when cells are deprived of oxygen, glucose levels, and an inflammatory reaction. VEGF is required for normal growth in vascularization during embryonics, cyclical blood vessel growth in female reproductive organs, and capillary formation during wound repair [20]. The role of VEGF as a regulator of angiogenic processes in early pregnancy is quite high. However, in the human placenta, VEGF levels are not consistent in the condition of preeclampsia [21]. The differences may be related to several factors, including the VEGF isoform that has been examined, it is due to the VEGFA gene that has 25 variants that produce at least 19 protein codes with wide variations

in bioavailability and biological activity [22]. A study showed based on the expression of mRNA and VEGFA protein its specific variant with 165 amino acids (VEGF165) which is one of the most biologically active isoforms. The results of the study indicated that the mRNA was higher for VEGF165 only for placentas from early onset preeclampsia compared to placentas of normal pregnancy [23].

## Conclusion

The study reported that urine protein levels of 2+ and 3+ are greater in early onset than late onset preeclampsia. The concentration of VEGF in the early onset of preeclampsia is lower than the late onset of preeclampsia.

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