



RESEARCH ARTICLE

The Role of Endothelial Nitric Oxide in Preeclampsia Onset

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Background: Preeclampsia is a pregnancy-specific hypertensive disorder associated with significant maternal and fetal morbidity and mortality. Endothelial dysfunction plays a critical role in the pathogenesis of preeclampsia, with nitric oxide (NO) being a key regulator of vascular tone and endothelial function. This study aims to investigate the relationship between endothelial nitric oxide (eNOS) levels and the onset of preeclampsia.

Methods: A cross-sectional study was conducted on pregnant women diagnosed with severe preeclampsia and receive treatment at Dr. M. Djamil Hospital, Padang. After met the inclusion criteria and do not have exclusion criteria, demographic and clinical data was taken. Maternal plasma concentrations of eNOS were determined by ELISA. Non-parametric statistics were used for analysis.

Results: The study evaluated the levels of endothelial nitric oxide synthase (eNOS) in two groups of preeclampsia patients: those with Early Onset Preeclampsia (EOP) and Late Onset Preeclampsia (LOPE). The median eNOS levels were 50.43 pg/ml for the EOP group (range: 28.72 - 232.78 pg/ml) and 54.76 pg/ml for the LOPE group (range: 15.39 - 235.70 pg/ml). A Mann-Whitney U test revealed a p-value of 0.303, indicating no statistically significant difference in eNOS levels between the two groups. This suggests that the timing of preeclampsia onset does not significantly influence eNOS levels, providing insights into the disease's mechanisms.

Conclusion: eNOS levels in the early preeclampsia (EOP) group were found to be lower compared to the late preeclampsia (LOPE) group. Although there was a difference in eNOS levels between the two groups, the difference was not statistically significant.

Keywords: preeclampsia; endothelial dysfunction; nitric oxide; pregnancy; hypertensive disorders

INTRODUCTION

Preeclampsia remains a leading cause of maternal and perinatal morbidity and mortality worldwide, contributing significantly to adverse pregnancy outcomes. Among the various factors implicated in its pathogenesis, endothelial nitric oxide synthase (eNOS) plays a crucial role. In preeclamptic patients, the expression of antiangiogenic factors, including soluble endoglin (sEng), increases, while proangiogenic factors such as vascular endothelial growth factor (VEGF) decrease. This imbalance leads to extensive endothelial damage and impaired placentation.^{1,2}

The inhibition of eNOS activity by sEng, which binds to transforming growth factor-beta (TGF- β), activates platelets and enhances leukocyte adhesion, further exacerbating endothelial dysfunction. This chain of events is pivotal in the development of preeclampsia. In particular, the differences in eNOS levels have been linked to the pathophysiological distinctions between early onset preeclampsia (EOP) and late onset preeclampsia (LOPE). EOP is often characterized by significant placental abnormalities and a clear association with reduced eNOS levels, while LOPE appears to be more influenced by maternal factors without marked placental involvement.^{3,4}

Endothelial nitric oxide synthase (eNOS) has emerged as a valuable marker for preeclampsia (PE) due to its association with nitric oxide (NO) bioavailability, which is critical in regulating blood vessel tone and placental perfusion. The study by Kaihara et al. observed that eNOS plasma concentrations are notably lower in preeclamptic women than in those with gestational hypertension, healthy pregnancies, or non-pregnant women. This pattern underscores eNOS's role in vascular health and highlights how its deficiency might contribute to endothelial dysfunction, a hallmark of PE.⁴

Abnormal placentation, common in PE, is marked by inadequate trophoblast invasion into the spiral arteries, which in turn promotes oxidative stress, hypoxia, and reduced placental perfusion. This cascade is partially driven by decreased NO availability, as NO production relies on eNOS. In a healthy pregnancy, eNOS facilitates NO production, helping to reduce uteroplacental resistance and support fetal development. Reduced eNOS disrupts this NO balance, predisposing individuals to the endothelial dysfunction and placental abnormalities seen in PE.⁴

While placental and genetic maternal factors influence PE development, eNOS levels were similarly reduced across both early- and late-onset PE, suggesting that endothelial dysfunction from low NO bioavailability contributes to both types. The mechanism diverges slightly: in early-onset PE, placental dysfunction due to eNOS reduction directly affects endothelial function; in late-onset PE, maternal metabolic factors (e.g., obesity, hypertension) increase oxidative stress, further diminishing eNOS and exacerbating endothelial dysfunction. This finding supports eNOS as a marker reflecting the shared pathophysiological underpinning across PE subtypes.⁵

This study aims to explore the relationship between eNOS levels and the onset of preeclampsia, hypothesizing that significant differences in eNOS levels will be observed between EOP and LOPE, contributing to our understanding of the underlying mechanisms of this complex condition.

METHODS

This study used a quantitative research method with a cross-sectional design. Pregnant women diagnosed with severe preeclampsia and receive treatment at Dr. M. Djamil Hospital, Padang who met the inclusion criteria and did not have exclusion criteria was included in the study. Inclusion criteria included agreed to participate, age ≥ 18 years, singleton pregnancy, diagnosed with preeclampsia at less than 34 weeks of gestation for EOP and after 34 weeks for LOPE, and had uncomplicated preeclampsia which no end organ damage was found. Exclusion criteria included being treated with different management from usual procedures at Dr. Hospital. M. Djamil; died, or discharge against medical advice or was referred to another hospital before data collection was completed; dropped out from the study before data collection is complete; Research materials are damaged, cannot be assessed, or incomplete data; had major fetal morphological abnormalities discovered through examination.

Demographic data was taken during anamnesis. Clinical and laboratory data are obtained from physical and laboratory examination. As much as 5ml of venous blood was taken from the median cubital vein to examine serum levels of eNOS using the ELISA method.^{1,2} Complete blood count, renal function, liver function, electrolyte also assessed during laboratory examination.

To assess differences in serum levels of eNOS between in both groups, the t-test was used if the data were normally distributed, with a significance limit of $p=0.05$. If one or both groups of data are not normally distributed, the analysis used is a nonparametric test.

RESULT

The study aimed to evaluate and compare the levels of endothelial nitric oxide synthase (eNOS) between two groups of preeclampsia patients: those with **Early Onset Preeclampsia (EOP)** and **Late Onset Preeclampsia (LOPE)**. eNOS is an enzyme responsible for producing nitric oxide, a molecule involved in vasodilation and maintaining endothelial function. Given the potential role of eNOS in the pathophysiology of preeclampsia, understanding its levels in different types of preeclampsia may provide insights into disease mechanisms.

eNOS Levels in EOP and LOPE

Table 1. Characteristics of subjects participated in this study

Characteristic	EOP		LOPE	
	Mean $\pm$ SD	Min - Max	Mean $\pm$ SD	Min - Max
Age (years)	31.80 $\pm$ 5.57	21- 42	30.47 $\pm$ 6.73	15 - 43
BMI (kg/m ²)	26.28 $\pm$ 4.85	18.36 – 39.6	27.23 $\pm$ 5.88	20 – 40.15
Gestational age (weeks)	29.42 $\pm$ 3.41	20 – 33	36 $\pm$ 2.07	30 – 40
Parity				
Primiparous (%)	25 (62,5)		31 (77,5)	
Multiparous (%)	15 (37,5)		9 (22,5)	
eNOS (pg/ml)	59.64 $\pm$ 32.64	28.72 – 232.78	68.95 $\pm$ 44.11	15.39 – 235.70
HB (g/dl)	12.49 $\pm$ 1.7	6.8 – 16.7	12.42 $\pm$ 1.47	9.40 – 15
Leu (/mm ³)	15,651 $\pm$ 5,460	5,510 – 29,860	14,648 $\pm$ 6,537	6,840 – 34,760
Thrombocyte (/mm ³)	168,300 $\pm$ 84,725	30,000 – 387,000	244,875 $\pm$ 92,458	135,000 – 513,000
RBG (mg/dL)	117.72 $\pm$ 65.54	57 - 476	106.02 $\pm$ 44.39	54 – 248
AST (μ /l)	61.32 $\pm$ 68.06	5 - 337	34.31 $\pm$ 54.61	2.7 – 307
ALT (μ /l)	35.97 $\pm$ 38.07	4 - 149	29 $\pm$ 48.96	5 – 259
Ureum (mg/dL)	44.62 $\pm$ 58.56	4 - 377	23.47 $\pm$ 15.78	4 – 72
Creatinine (mg/dL)	1.8 $\pm$ 1.8	0.3 - 7	1.61 $\pm$ 2.01	0.4 – 8
PT (second)	10.51 $\pm$ 3.76	8.5 – 28.5	9.32 $\pm$ 0.66	8.09 – 10.6
APTT (second)	28.07 $\pm$ 6.72	9.7 – 45.2	25.55 $\pm$ 3.42	14.02 – 32.8
Na (mmol)	136.02 $\pm$ 3.94	119 - 141	136.35 $\pm$ 10.95	75 – 144
K (mmol)	4.01 $\pm$ 0.61	2.8 – 5.8	3.84 $\pm$ 0.64	2.6 – 5.1
Cl (mmol)	105.95 $\pm$ 4.54	86 - 111	105.92 $\pm$ 4.23	4 - 116

As shown in Table 1, the median eNOS level in the EOP group was 50.43 pg/ml, with a minimum value of 28.72 pg/ml and a maximum value of 232.78 pg/ml. In comparison, the median eNOS level in the LOPE group was 54.76 pg/ml, ranging from 15.39 pg/ml to 235.70 pg/ml.

- **EOP (Early Onset Preeclampsia):**
 - Median eNOS level: 50.43 pg/ml
 - Range: 28.72 - 232.78 pg/ml
- **LOPE (Late Onset Preeclampsia):**
 - Median eNOS level: 54.76 pg/ml
 - Range: 15.39 - 235.70 pg/ml

Statistical Analysis

Table 2. eNOS, sEng and 25-Hydroxy Vitamin D levels in Early onset preeclampsia and Late onset preeclampsia

Variable	EOP	LOPE	P value
eNOS (pg/ml)	59.65 ± 32.65	68.95 ± 44.10	^a 0.303
sEng (pg/ml)	0.09 ± 0.09	0.11 ± 0.09	^a 0.468
25-Hydroxy Vitamin D (ng/ml)	25.02 ± 12.04	32.65 ± 20.02	^a 0.164

^aMann Whitney

To assess whether there was a statistically significant difference in eNOS levels between the two groups, a **Mann-Whitney U test** was performed. This non-parametric test is appropriate for comparing medians between two independent groups when the data may not follow a normal distribution. The result of the test yielded a **p-value of 0.303**, which is greater than the conventional threshold for statistical significance ($p < 0.05$).

Interpretation of Findings

The p-value of **0.303** indicates that there is **no statistically significant difference** in eNOS levels between patients with Early Onset Preeclampsia (EOP) and those with Late Onset Preeclampsia (LOPE). This finding suggests that the timing of the onset of preeclampsia (whether early or late) does not appear to significantly affect the levels of eNOS in these patient groups.

DISCUSSION

In this study, no significant difference in eNOS levels was found between the Early Onset Preeclampsia (EOP) and Late Onset Preeclampsia (LOPE) groups, indicating that eNOS levels were reduced in both EOP and LOPE. This finding is consistent with previous studies. A 2023 study by Kaihara et al. involving 417 subjects with preeclampsia (PE) found that non-pregnant women and those with gestational hypertension had lower plasma eNOS concentrations compared to healthy pregnant women, with eNOS levels being further reduced in preeclampsia patients. However, similar to this study, they did not find a statistically significant difference in eNOS levels between early and late onset preeclampsia ($p > 0.05$).⁶⁻⁸

Additionally, a study by Laskowska et al. in 2013 also found no significant difference in eNOS levels between EOP and LOPE.⁹ This study included 62 EOP patients, 53 LOPE patients, and 65 healthy pregnant women. The reported eNOS levels were slightly higher than in our findings but did not show significant differences based on preeclampsia onset ($p = 0.64$).

Shaheen et al. (2021) conducted a study on 600 women, including 188 PE patients and 112 with severe PE, and found that nitric oxide (NO) levels were reduced in all PE groups.¹⁰ This study also highlighted that mutations in the Glu298Asp protein destabilized eNOS proteins, leading to lower NO concentrations. The average gestational age in the study was around 33.12 weeks,

showing reduced eNOS levels in both EOP and LOPE, which contributed to lower NO availability.¹¹⁻¹⁴ The pathophysiology of PE remains unclear, but it is thought to involve abnormal placentation, impaired trophoblast invasion, oxidative stress, and endothelial dysfunction. Reduced eNOS bioavailability is a key factor, as it decreases nitric oxide (NO) production, which is crucial in maintaining vascular function during pregnancy. Impaired NO production can lead to fetoplacental blood flow issues and abnormal placentation, making it a significant factor in both types of PE.¹⁵⁻¹⁷ Although EOP and LOPE are believed to have different underlying mechanisms—EOP being linked to placental defects and LOPE to maternal factors like obesity and hypertension—reduced plasma eNOS concentrations appear to be common in both types of preeclampsia. In EOP, decreased eNOS lowers NO bioavailability, leading to endothelial dysfunction and abnormal placentation. In LOPE, maternal comorbidities contribute to increased reactive oxygen species (ROS) and oxidative stress, which further reduce eNOS levels.¹⁸⁻²¹

CONCLUSION

eNOS levels in the early preeclampsia (EOP) group were found to be lower compared to the late preeclampsia (LOPE) group. Although there was a difference in eNOS levels between the two groups, the difference was not statistically significant.

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