# The Correlation of L-citrulline Levels with Blood Pressure in Severe Preeclampsia

*by* Afriwardi Afriwardi

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RESEARCH ARTICLE

# The Correlation of L-citrulline Levels with Blood Pressure in Severe Preeclampsia

Hudila Rifa Karmia[1], Afriwardi[2], Hirowati Ali[3], Johanes Cornelius Mose[4], Yusrawati[4,*]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Andalas, Jl. Perintis Kemerdekaan, Padang 25127, Indonesia
[2]Faculty of Medicine, Universitas Andalas, Jl. Perintis Kemerdekaan, Padang 25127, Indonesia
[3]Biochemistry Division, Faculty of Medicine, Universitas Andalas, Jl. Perintis Kemerdekaan, Padang 25127, Indonesia
[4]Fetomaternal Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Andalas, Jl. Perintis Kemerdekaan, Padang 25127, Indonesia

*Corresponding author. E-mail: yusrawati65@med.unand.ac.id

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## Abstract

**BACKGROUND:** Based on 'vascular disorder of pregnancy' terminology, preeclampsia primarily was not a hypertensive disorder, but a vascular disorder (general vasospasm) in pregnancy due to idiopathic etiology. The overall incidence of preeclampsia was 5-14% of all pregnancies. One of the substances responsible for regulating vascular tone is nitric oxide (NO), which produced in endothelial blood vessels. NO and L-citrulline are produced altogether by the reaction between L-arginine and oxygen. L-citrulline levels reflected NO production. This study was aimed to assess the correlation between the L-citrulline level and blood pressure in severe preeclampsia.

**METHODS:** This cross-sectional study was done in Dr. M. Djamil Central General Hospital, Padang, Indonesia The sample size was 36 samples of pregnant women with severe preeclampsia and had yet been given antihypertensive therapy. Sampling was done by consecutive sampling from Obstetrics and Gynecology Division. After maternal examination and measurements L-citrulline levels of cubital venous blood by using enzyme-linked immunosorbent assay (ELISA) method, Pearson correlation was performed to assess the relationship between variables for normally distributed data and Spearman's correlation for abnormal distribution data with significance level $p<0.05$.

**RESULTS:** Means of L-citrulline levels, systolic blood pressure, diastolic blood pressure, and arterial pressure (MAP) were 87.21 nmol/mL, 179.4 mmHg, 108.3 mmHg, and 132.1 mmHg, respectively. Correlation of the L-citrulline level with systolic blood pressure, diastolic blood pressure, and MAP were -0.08, -0.175, and -0.136 ($p>0.05$), respectively.

**CONCLUSION:** L-citrulline levels had no correlation with blood pressure in severe preeclampsia.

**KEYWORDS:** L-citrulline levels, blood pressure, severe preeclampsia

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## Introduction

Preeclampsia is one of maternal mortality main causes until today besides bleeding and infection. The overall incidence of preeclampsia was 5-14% of all pregnancies. It was later found out that the incidence of preeclampsia is about 15.9% of all maternal deaths in the United States and was the greatest causes of perinatal morbidity and mortality. Its exact etiology which had yet been elucidated had caused primary prevention and casual therapy to be difficult, therefore controlling its morbidity and mortality was proven to be a challenge.(1)

Preeclampsia had been found worldwide with a varied incidence between 2%-8% (2), with a mean of 4.6% (1). While in developing countries, this was varied between 1.8%-16.7%.(3) The incidence reported in Indonesia was still dominated by the hospital-based reports. Prevalence of

preeclampsia in Dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, Indonesia was 16.6% (4), while in Dr. M. Djamil Central General Hospital was 7.1% (5).

Nitric oxide (NO) was produced from L-Arginine and $O_2$ reaction catalyzed by NO synthase (NOS) enzyme. Another product of this reaction was L-citrulline. NOS enzyme activity stimulates NO production during pregnancy. The up-regulation of NO roles in pregnancy was due to the increase of estrogen level.(6) Physiologic disturbance of NO was the main etiology cause of preeclampsia, therefore, the administration of exogenous NO was being an interesting treatment option to be done.(7) Disturbances in NO production caused the imbalance of reactive oxygen species (ROS) and NO which subsequently causing dysfunction of vasodilation, therefore, blood pressure tends to increase.(6)

The measurement of NO levels was so difficult to be done due to its extremely short half-life. NO was immediately reduced to nitrite and nitrate anion (both were called NO metabolites). Measurement of both metabolites was used as a surrogate measurement of NO production by endothelial NOS enzymes.(8) Then, NO and L-citrulline were both end products of L-arginine and $O_2$ reaction catalyzed by NOS enzyme, bearing in mind that if this reaction was adequately produced by enough NO and L-citrulline, vice versa. L-citrulline levels reflected NO production.(9) Based on the facts above, this study was done to assess the correlation between L-citrulline level and blood pressures in severe preeclampsia.

## Methods

### Samples Selection and Recruitment

This research was an observational analytic study with cross sectional study design, that was conducted during four month from November – February 2019. This study was assessing correlation of L-citrulline levels with blood pressure before administration of antihypertensive and anticonvulsant drugs. As determined with Federer formula, a sample size of 36 samples was done by consecutive sampling of preeclampsia case admitted to hospital and had no antihypertensive and anticonvulsive drugs which had met inclusion criteria and passed exclusion criteria. The inclusion criteria in this study were all women with preeclampsia, had no antihypertensive and anticonvulsive drugs before. Sampling was done by consecutive sampling, taken from Obstetrics and Gynecology Division of Dr. M. Djamil Central General Hospital, Padang, Indonesia.

All patients signed the informed consent and filled the history questionnaire before the clinical examination and obstetric examination were performed. This research has passed the ethical clearance test from Ethics Committee, Faculty of Medicine, Universitas Andalas (No. 362/KEP/FK/2018).

### Clinical and Obstetric Examination

After signing informed consent, history, clinical examination and obstetric examination, and ultrasound were performed. Blood pressure was obtained and then mean arterial pressure (MAP) was calculated.

### Enzyme-linked Immunosorbent Assay (ELISA) Examination

Three mL of cubital venous blood was withdrawn to measure L-citrulline levels in serum using Cusabio reagent (Catalogue #CSB-E13832h, Houston, USA) and Bio-Rad electrophoresis with ELISA method.

### Statistical Analysis

Statistical analysis was performed using SPSS (IBM Corporation, New York, USA). Univariate analysis was used to observe the characteristics of respondents. Bivariate analysis was done using Pearson correlation test if data distribution was normal and Spearman correlation test was used when data distribution was not normal with significance level $p<0.05$. The normality test was assessed using Shapiro-Wilk test (total sample in group less than 50).

## Results

The maternal age was ranged between 20-44 years old with patients mean age was 31.9 years old. The mean of gestational age was 33.6 weeks. Table 1 showed that most of the samples were multipara (72.2%) and more than half was a late onset of preeclampsia (52.8%).

The mean of subjects' L-citrulline level was 87.21 nmol/mL, meanwhile the mean of systolic blood pressure, diastolic blood pressure, and MAP were 179.4 mmHg, 108.3 mmHg, and 132.1 mmHg, respectively (Table 2).

Table 3 showed the correlation of L-citruline level and blood pressure. L-citruline level had a weak negative correlation with systolic blood pressure, diastolic blood pressure, and MAP, respectively. L-citruline level had no significant correlation with systolic blood pressure, diastolic blood pressure, and MAP, whereas these three maternal outcome parameters had $p>0.05$.

**Table 1. Characteristics of parity and preeclampsia onset.**

| Characteristic | n | % |
|---|---|---|
| **Parity** | | |
| Primipara | 10 | 27.8 |
| Multipara | 26 | 72.2 |
| Total | 36 | 100 |
| **Preeclampsia onset** | | |
| Early | 17 | 47.2 |
| Late | 19 | 52.8 |
| Total | 36 | 100 |

## Discussion

Mean L-citrulline levels in this study was 87.21 nmol/mL, or 87.21 µmol/L. This finding was twice of a healthy person, hat is 35.19 µmol/L.(8) This finding was also higher than previous report, where levels of severe preeclampsia from 17 subjects was 44.1 µmol/L.(9) Plasma levels of L-citrulline, which reflect NO production, are significantly higher in women with severe preeclampsia than in normotensive pregnant women.(9) High L-citrulline level found in severe preeclampsia in this study seemed in contrast with the hypothesis that L-citrulline levels in preeclampsia should be lower than in normal healthy persons. This fact could happen since this L-citrulline level reflected high production of NO, and high production of NO is a compensation phenomenon to the increased synthesis and release of vasoconstrictor and platelet-aggregating agents in severe preeclampsia.(9) The increase of synthesis and release of vasoconstrictor and platelet-aggregating agents mainly occurred in placental preeclampsia, therefore it is possible that (most of) preeclampsia cases being this study subjects had main etiopathogenesis of placental factor and not a vascular factor.

In this study, the mean systolic pressure was 179.4 mmHg and diastolic pressure was 108.3 mmHg. This findings indicated that (most of) these study subjects were categorized as severe preeclampsia, fulfilling criteria systolic blood pressure >110 mmHg, and not due to diastolic pressure of >110 mmHg, which means that systolic pressure increase was more prominent than diastolic pressure in this study subjects; or in other words, there was no large increase in diastolic pressure.

NO had crucial roles in endothelial regulation, controlling blood pressure, and cardiovascular haemostasis. (10) NO caused vascular relaxation and suppressed thrombocyte adhesion to vascular endothelial.(7,11) This effect was mediated by 3',5'-cyclic guanosine monophosphate (cGMP) produced by soluble guanylyl cyclase, cGMP activated protein kinase A (PKA) and protein kinase G (PKG). Activated PKA and PKG induced smooth muscle relaxation by attenuating myosin light chain kinase activity and enhancing myosin light chain phosphatase activity (6,12), which resulted in the decrease of muscle contraction strength through two mechanisms, *i.e.*, decreased $Ca^{2+}$ level or decreased sensitivity to $Ca^{2+}$ (13).

One substance that plays an important role in regulating blood pressure generated is endothelial-derived endothelial nitric oxide (eNO), or also knows as NOS3. NO has a crucial role in the regulation of endothelial, controlling blood pressure, and cardiovascular hemostasis (10) by relaxing vascular and suppress platelet adhesion to vascular endothelium (11). The disorder can result in primary endothelial NO production is not enough that the vasoconstrictor factors are more dominant and higher blood pressure. Potential primary hypertension due to endothelial dysfunction can manifest in pregnancy and is called 'vascular disorder of pregnancy'.(13)

Diastolic pressure was more depicting base vascular tone, and NO plays a role in base vascular tone regulation as vasodilator substance (causing vascular relaxation).(11) NO large increase in diastolic pressure could be understood as a condition where the decrease of NO production was not

**Table 2. L-citrulline levels and blood pressure.**

| Variable | Mean±SD | Min | Max |
|---|---|---|---|
| L-citrulline levels (nmol/mL) | 87.21±57.79 | 1.2 | 156.3 |
| Systolic blood pressure (mmHg) | 179.4±24.8 | 160 | 270 |
| Diastolic blood pressure (mmHg) | 108.3±15.8 | 90 | 150 |
| Mean arterial pressure (mmHg) | 132.1±17.5 | 113 | 190 |

**Table 3. Correlation of L-citruline level and blood pressure.**

| L-citrulline Level | Systolic Blood Pressure | Diastolic Blood Pressure | Mean Arterial Pressure |
|---|---|---|---|
| r | -0.08 | -0.175 | -0.136 |
| p | 0.786 | 0.309 | 0.427 |
| $r^2$ | 0.006 | 0.031 | 0.018 |

the main underlying cause of severe preeclampsia in this study subjects, in short, the main etiopathogenesis was not a vascular factor.

This study result did not prove that there was a correlation between L-citrulline levels with blood pressure. Another possibility to explain this fact was that the increase of blood pressure (occurrence of preeclampsia) in this study sample pathogenetically was not due to lack of NO production, lack of NOS3 enzyme activity, or due to downregulation of NOS gene due to polymorphism of NOS3 gene (especially at G-984 and T-786).(10) There is a relationship between the presences of the gene NOS3 Single-nucleotide polymorphism T-786 (SNP T-786) with the occurrence of preeclampsia.(14) This could be elucidated if a further study was done in this study subjects to examine the association between NOS3 gene/ polymorphism of the NOS3 gene, NOS3 enzyme level, L-citrulline level, and blood pressure. If those studies were also proven to be wrong, it could be concluded that the main etiopathogenesis of preeclampsia in this study subjects was not due to vascular factor.

## Conclusion

This study proved that L-citrulline levels doesn't correlate with severe preeclampsia. Increase of blood pressure was not due to lack of NO production that reflected by L-citrulline level.

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## References

1. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013; 170: 1-7. doi: 10.1016/j.ejogrb.2013.05.005.
2. Jeyabalan A. Epidemiology of preeclampsia: impact of obesity. Nutr Rev. 2013; 71: 18-25.
3. Osungbade KO, Ige OK. Public health perspectives of preeclampsia in developing countries: implication for health services strengthening. J Pregnancy. 2011; 2011:481095. doi: 10.1155/2011/481095.
4. Perpustakaan Fakultas Kedokteran Universitas Indonesia. Prevalensi preeklampsia berat di Rumah Sakit Ciptomangunkusumo. Jakarta: Perpustakaan Fakultas Kedokteran Universitas Indonesia; 2014.
5. Serudji J. Minimizing the occurrence and complications of preeclampsia: prevention and prediction. In: Pertemuan Ilmiah Tahunan (PIT) Fetomaternal XIII 2012 Mar 10-14, Palembang. Palembang: PIT Fetomaternal; 2012.
6. Matsubara K, Higaki T, Matsubara Y, Nawa A. Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. Int J Mol Sci. 2015; 16: 4600-14.
7. Johal T, Lees CC, Everett TR, Wilkinson IB. The nitric oxide pathway and possible therapeutic options in pre-eclampsia. Br J Clin Pharmacol. 2014; 78: 244–57.
8. Hess S, Baker G, Gyenes G, Tsuyuki R, Newman S, Le Melledo J. Decreased serum L-arginine and L-citrulline levels in major depression. Psychppharmacology. 2017; 234: 3241-7.
9 Benedetto C, Marozio L, Neri I, Giarola M, Volpe A, Facchinetti F. Increased L-citrullinel-arginine plasma ratio in severe preeclampsia. Obstet Gynecol. 2000; 96: 395-9.
10. Zeng F, Zhu S, Wong MC, Yang Z, Tang J, Li K, *et al.* Associations between nitric oxide synthase 3 gene polymorphisms and preeclampsia risk: a meta-analysis. Sci Rep. 2016; 6: 23407. doi: 10.1038/srep23407.
11. Rai H, Parveen F, Kumar S, Kapoor A, Sinha N. Association of endothelial nitric oxide synthase gene polymorphisms with coronary artery disease: an updated meta-analysis and systematic review. PLoS One. 2014; 9: e113363. doi: 10.1371/journal.pone.0113363.
12. Treuer AV and Gonzalez DR. Nitric oxide synthases, s-nitrosylation and cardiovascular health: from molecular mechanisms to therapeutic opportunities. Mol Med Rep. 2015; 11: 1555-65.
13. Berhan Y. No hypertensive disorder of pregnancy; no preeclampsia-eclampsia; no gestational hypertension; no. Hellp syndrome. Vascular disorder of pregnancy speaks for all. Ethiop J Health Sci. 2016; 26: 177-86.
14. Chen M, Tang W, Hou L, Liu R, Dong Z, Han X, *et al.* Tumor necrosis factor (TNF) -308G> A, nitric oxide synthase 3 (NOS3) + 894G> T polymorphisms and migraine risk: a meta-analysis. PlosOne. 2015; 10: e0129372. doi: 10.1371/journal.pone.0129372.

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