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Comparison of Serum Vitamin D (25(OH)D) Levels in Pregnancies with Preeclampsia, Severe Preeclampsia, and Normal Blood Pressure in the Third Trimester

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ABSTRACT

Background & Objective: Preeclampsia is a complication characterized by new-onset hypertension after 20 weeks of gestation, increasing the risk of maternal and neonatal morbidity and mortality, including renal failure, stroke, and cardiac arrest. Known risk factors include nulliparity, maternal age>35 years, chronic hypertension, and vitamin D deficiency. Vitamin D (25(OH)D) contributes to endothelial function, placental development, and oxidative stress regulation, potentially influencing preeclampsia risk. This study aimed to compare serum 25(OH)D levels pregnant women with normal blood pressure, preeclampsia, and severe preeclampsia.

Materials & Methods: This cross-sectional study was conducted on 60 pregnant women, divided into three groups: normal blood pressure, preeclampsia, and severe preeclampsia.

Results: Serum 25(OH)D levels decreased progressively with increasing preeclampsia severity. Mean 25(OH)D levels were 21.6 ng/mL in the normal group, 15.5 ng/mL in preeclampsia group, and 12.3 ng/mL in the severe preeclampsia group. Statistical analysis showed a significant association between low 25(OH)D levels and increased preeclampsia severity (P=0.001). Spearman correlation demonstrated a strong negative correlation between 25(OH)D levels and disease severity (r= -0.663, P=0.001).

Conclusion: There is a significant difference in 25(OH)D levels among pregnancies with normal blood pressure, preeclampsia, and severe preeclampsia. Lower vitamin D status is significantly correlated with increased blood pressure severity.

Keywords: 25(OH)D levels, Pregnancy with normal blood pressur, Severe preeclampsia, Preeclampsia

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1. Introduction

Preeclampsia is a hypertensive disorder of pregnancy characterized by elevated blood pressure as a manifestation (1). According to The American College of Obstetricians and Gynecologists (ACOG), preeclampsia is defined as new-onset hypertension after 20 weeks of gestation accompanied by organ dysfunction (2).

The prevalence of preeclampsia in pregnancy in the United States is 3-5% (3), while in Asia it ranges from 2-10%, with Southeast Asian countries reporting 4-6%. In Indonesia, the incidence reaches 128,273 cases annually (5.3%) (4,5). Globally, preeclampsia and eclampsia contribute to approximately 50,000 maternal deaths per year, accounting for 26% of maternal mortality in the Latin America (6). The biggest cause of death from preeclampsia is eclampsia (7). Early-onset preeclampsia occurs in 0.38% of pregnancies, while late-onset preeclampsia occurs in 2.72% (8). Several factors including obesity, employment conditions and socioeconomic status are associated with increased risk (9-12).

Vitamin D is a steroid hormone involved in cell proliferation and differentiation (13), including reduction of pro-inflammatory cytokines such as IL6 (14). Vitamin D deficiency among pregnant women reaches 63%, while the prevalence of vitamin D insufficiency and hypovitaminosis D is 25% and 78%, respectively. The average serum vitamin D level among pregnant women in Indonesia was reported to be 40.59 nmol/L, which is below the deficiency

threshold of 50 nmol/L **(15)**. Another study from Indonesia similarly demonstrated a very high prevalence of vitamin D deficiency, with a mean serum level of 11.3 ng/mL **(16)**.

A meta-analysis found that vitamin D (25(OH) deficiency increases the risk of preeclampsia (2.09-fold), preterm birth (1.58-fold), and low birth weight (1.52-fold) **(17)**. However, recent systematic reviews show inconsistent findings, with some studies supporting an association between vitamin D deficiency and preeclampsia, while others do not **(18)**.

This study aimed to evaluate differences in serum 25(OH)D levels in pregnancies with normal blood pressure, preeclampsia, and severe preeclampsia.

2. Materials and Methods

This cross-sectional study was conducted at Airlangga University Hospital in 2024. Subject inclusion criteria were pregnant women of any age with normal blood pressure, preeclampsia, or severe preeclampsia.

Preeclampsia (PE) was defined as Gestational age≥20 weeks; no previous history of hypertension; systolic blood pressure≥140 mmHg and <160 mmHg; diastolic blood pressure≥90 mmHg and <110 mmHg; and proteinuria≥300 mg/ 24 hours or ≥1+ on dipstick examination.

Severe Preeclampsia was defined as meeting the criteria for preeclampsia along with at least one of the following: systolic blood pressure≥160 mmHg or diastolic blood pressure≥110 mmHg with proteinuria≥1+ on dipstick/boiled protein; systolic blood pressure≥140 mmHg or diastolic blood pressure≥90 mmHg accompanied by platelet count<100,000/µL; serum creatinine>1.1 mg/dL; elevated liver enzymes (more than twice the normal level); Pulmonary oedema; symptoms such as headache, visual disturbances, or epigastric pain (impending eclampsia); fetal growth restriction; third-trimester pregnancy (gestational age 28-42 weeks at the time of delivery); and singleton intrauterine pregnancy.

Exclusion Criteria: refuses to participate, history of DIC, liver disease, haematological and coagulation disorders, kidney disease, chronic hypertension, cardiovascular disease, or urinary tract disease.

Serum 25(OH)D levels were measured using Chemiluminescent Microparticle Immunoassay (CMIA) with ARCHITECT 25-OH Vitamin D 5P02 (Abbott Laboratories; Illinois; United States of America). In order to measure 25(OH)D concentration using mass spectrometry, serum was mixed with anti-vitamin D IgG reagent and then washed out several times.

Levels were classified as:

- Deficient: <20 ng/ml
- Insufficient: 20-29.9 ng/ml
- Adequate: 30-100 ng/ml.

A total of 60 subjects were included (20 per group). All participants provided informed consent.

Statistical Analysis

Categorical variables were presented as percentages, and numerical variables as mean±SD or median depending on distribution.

- Chi-square test was used for categorical variables.

- One-Way Anova was used for normally distributed numerical variables.

- Spearman correlation assessed the relationship between 25(OH)D levels and disease severity. A P-value<0.05 was considered statistically significant. Analyses were performed using SPSS version 26 (IBM, USA).

3. Results

In this study, 60 samples meeting the inclusion criteria were collected and divided into three groups: 20 pregnant women with normal blood pressure, 20 with preeclampsia, and 20 with severe preeclampsia. All subjects underwent anamnesis, laboratory testing, and physical examination. Most participants (81.6%) were younger than 35 years. The youngest subject was 19 years old, and the oldest was 42 years old. The characteristics of the study population are summarized in Table 1.

The mean maternal age in the normal blood pressure group was 27.7±4.8 years, in the preeclampsia group 29.4±5.9 years, and in the severe preeclampsia group 29.5±6.2 years. The youngest subjects were 19 years old in both the normal and preeclampsia groups, whereas the youngest subject in the severe preeclampsia group was 22 years old. The oldest subjects were 37 years old in the normal blood pressure group and 42 years old in both the preeclampsia and severe preeclampsia groups. There were no statistically significant differences in gestational age at delivery among the three groups. Most subjects in all groups delivered at>34 weeks of gestation.

The mean serum 25(OH)D level was 21.6±4.54 ng/mL in the normal blood pressure group, 15.5±4.59 ng/mL in the preeclampsia group, and 12.3±3.55 ng/mL in the severe preeclampsia group Table 2.

Median 25(OH)D levels were highest in the normal blood pressure group (20-25 ng/mL), lower in the preeclampsia group (15-20 ng/mL), and lowest in the severe preeclampsia group (10-15 ng/mL), indicating a progressive decline in vitamin D status with increasing disease severity.

Vitamin correlation analysis demonstrated a strong negative correlation between serum 25(OH)D levels and disease severity (r= -0.663), indicating that lower

vitamin D levels were associated with more severe preeclampsia Table 3.

The negative correlation coefficient confirms that higher blood pressure severity is associated with lower 25(OH)D concentrations.

Table 1. Descriptive data

Characteristics	Normal blood pressure (n=20)	Preeclampsia (n=20)	Severe preeclampsia (n=20)	P
Age, Mean±SD (years)	27.7±4.8	29.4±5.9	29.5±6.2	0.511
Gestational age (weeks) (n, %)				
Early onset 24-34	1(5)	1(5)	4(20)	0.189
Late onset >34	19(95)	19(95)	16(80)	
Education (n, %)				
Elementary school	1(5)	3(15)	1(5)	
Middle school	1(5)	2(10)	2(10)	
High school	15(75)	13(65)	14(70)	0.880
College educated	3(15)	2(10)	3(15)	
BMI (n, %)				
Normal	6(30)	4(20)	3(15)	
Overweight	9(45)	4(20)	8(40)	
Grade I obesity	4(20)	7(35)	5(25)	
Grade II obesity	0	4(20)	4(20)	0.346
Grade III obesity	1(5)	1(5)	0	
Parity (n, %)				
First pregnancy	10(50)	9(45)	9(45)	0.935
Multiple pregnancy	10(50)	11(55)	11(55)	
Employment Statis (n, %)				
Unemployed	11(55)	18(90)	15(75)	0.043*
Employed	9(45)	2(10)	5(25)	
Delivery method (n, %)				
vaginal birth	12(60)	15(75)	8(40)	0.079
C-section	8(40)	5(25)	12(60)	

Table 2. Correlation analysis of each group

Characteristics	Normal blood pressure (n=20)	Preeclampsia (n=20)	Severe preeclampsia (n=20)	P
25(OH)D levels Mean±SD (ng/mL)	21.6±4.54	15.5±4.59	12.3±3.55	0.01
25(OH)D status (n, %)				
Normal	0	0	0	
insufficient	12(60)	4(20)	0	
Deficient	8(40)	16(80)	20(100)	

Table 3. Correlation analysis of variables

	Normal blood pressure, preeclampsia, and severe preeclampsia	Systolic blood pressure	Diastolic blood pressure
25 (OH)D Level	r= -0.663	r= -0.606	r= -0.524
	P=0.01*	P=0.01*	P=0.01*
	N=60	N=60	N=60

4. Discussion

Vitamin D insufficiency and deficiency are highly prevalent among pregnant women in Asia, particularly in the South Asia. A systematic review and meta-analysis by Siddiqee et al., (2022), involving 20 studies and 7,804 participants from Bangladesh, India, Pakistan, and Nepal, reported a 65% prevalence of vitamin D insufficiency, with a mean serum level of 16.37 ng/ml. Pakistan had the highest prevalence (76%), followed by India (67%) and Bangladesh (64%) **(19)**.

In Indonesia, vitamin D deficiency among pregnant women reached 63%, with insufficiency and hypovitaminosis D reported at 25% and 78%, respectively. The average serum vitamin D level (40.59 nmol/L) is below the deficiency threshold (50 nmol/L) **(15)**. Another study from Jakarta by Noroyono et al. (2017) found severe deficiency, with mean first trimester level of 11.3 ng/mL, far below the normal threshold (≥30 ng/mL) **(20)**.

The present study demonstrated a significant association between decreased serum 25(OH)D levels and increased risk and severity of preeclampsia. These findings are consistent with the landmark study by Bodnar et al., (2007), which reported significantly lower 25(OH)D levels in American pregnant women with preeclampsia compared to those who had normotensive controls In Bodnar's study, serum 25(OH)D levels in preeclamptic women were 15% lower than those without preeclampsia (45.4 nmol/L vs. 53.1 nmol/L). The prevalence of vitamin D deficiency (<37.5 nmol/L) was also higher in the preeclampsia group (28.6% vs. 15.7%) **(21)**.

A study by Akbar et al, reported that serum calcium levels in the Early-Onset Preeclampsia (EOPE) group were significantly lower than those in the control group, while no significant difference was observed in 25(OH)D levels between the two groups. Furthermore, no correlation was found between serum calcium and 25(OH)D concentrations **(22)**.

Another study by Yeni et al., compared vitamin D levels between normotensive pregnant women and those with severe preeclampsia, demonstrating that serum vitamin D levels in severe preeclampsia were approximately two-fold lower than in the normal cohort **(23)**.

Regarding vitamin D (25(OH)D) status in the present study, 100% of subjects in the severe preeclampsia group exhibited vitamin D deficiency. In contrast, 60% of the normal blood pressure group had insufficiency and 40% had deficiency. In the preeclampsia group, 4% had insufficiency and 80% had deficiency. These findings indicate a markedly higher prevalence of vitamin D deficiency among women with severe preeclampsia. Previous studies support this observation, suggesting that low 25(OH)D levels may promote inflammation and endothelial dysfunction key pathogenetic mechanisms in preeclampsia **(24,25)**.

A meta-analysis evaluating the association between maternal 25(OH)D levels and preeclampsia confirmed that vitamin D deficiency increases risk of developing preeclampsia, whereas supplementation may reduce this risk. Thus, 25(OH)D deficiency represents an important modifiable factor influencing preeclampsia risk **(26)**.

Boxplot analysis in this study demonstrated that the severe Preeclampsia (PEB) group had a narrower distribution of 25(OH)D levels, indicating low variability and consistently low vitamin D status Figure 1. In contrast, the normal blood pressure group showed a wider distribution, reflecting greater variation in vitamin D levels. These findings further support the association between low 25(OH)D levels and both the occurrence and severity of **(21)**.

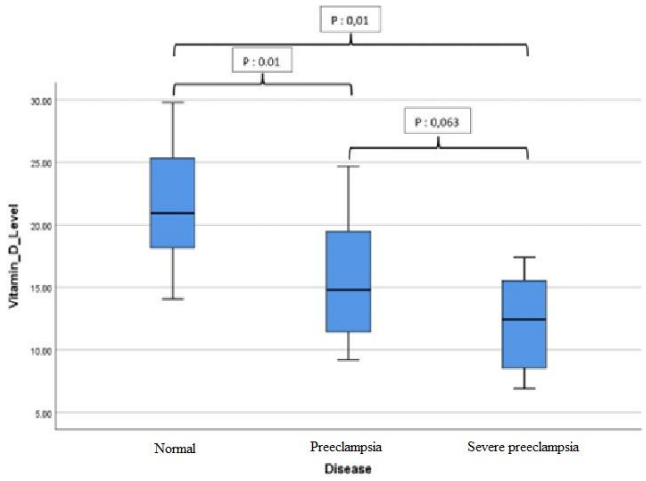


Figure 1. Boxplot analysis and Post hoc Analysis of 25(OH)D in three groups

The underlying mechanism may relate to the role of vitamin D in immune modulation and endothelial function. Vitamin D possesses anti-inflammatory properties and regulates immune responses, influencing vascular tone and blood pressure. A 2017 study by Purswani et al., highlighted that vitamin D deficiency may contribute to endothelial dysfunction and inflammation, both central to preeclampsia pathogenesis **(27)**.

A study was by Negm et al., (2022) in Egypt demonstrated significantly lower vitamin D levels in preeclamptic women compared to controls. Pregnant women with vitamin D deficiency (<20 ng/ml) were three times more likely to develop preeclampsia, and lower vitamin D levels were associated with greater disease severity **(28)**. In the present study, correlation analysis between 25(OH)D levels blood pressure revealed A statistically significant negative correlation of strong magnitude between serum 25(OH)D and systolic blood pressure (Pearson test) as well as A statistically significant negative correlation of moderate magnitude between serum 25(OH)D and diastolic blood pressure (Spearman test). This finding indicate that lower 25(OH)D levels are associated with higher systolic and diastolic blood pressure, supporting a potential role of vitamin D in blood pressure regulation.

This finding aligns with Pekkanen et al., (2015), who reported associations between low vitamin D levels and major cardiovascular risk factors, as well as structural changes in the heart, including impaired systolic and diastolic cardiac function **(29)**. Additionally, in vivo research by Ni et al., (2014) demonstrated that the Vitamin D Receptor (VDR) in endothelial cells is essential for maintaining endothelial function and regulating blood pressure, suggesting that VDR agonists may have therapeutic in cardiovascular diseases linked to endothelial dysfunction **(30)**. Forman et al., (2010) showed that low plasma 25(OH)D levels may upregulate the Renin-Angiotensin System (RAS) **(31)**.

The present study has several limitations. First, dietary habits were not assessed, despite evidence that Diet influences blood pressure, BMI, and preeclampsia risk. High salt intake has been shown to increase the risk of hypertension and preeclampsia **(32)**.

Second, employment status was not thoroughly evaluated. Employment may act as a confounding factor, and previous studies have suggested a possible association between employment conditions and increased preeclampsia risk **(33)**.

5. Conclusion

There is a significant difference in serum 25(OH)D levels among pregnancies with normal blood pressure, preeclampsia, and severe preeclampsia. Lower 25(OH)D levels are strongly associated with increased disease severity.

6. Declarations

Acknowledgments

The authors declare no acknowledgements.

Ethical Considerations

Ethical approval was obtained from the Universitas Airlangga Hospital Ethics Committee (No. 067/KEP/2024)

Authors' Contributions

Bayu Agung Sangkara Putra: Conceptualization, conceived and designed the analysis, collected the data, performed the analysis, wrote the paper. Muhammad Ilham Aldika Akbar: conceptualization, wrote and review the paper.

Clinical Significance

This study provides foundational evidence for future research using larger sample sizes and advanced

methodologies to further investigate the role of 25(OH)D in pregnancy and preeclampsia.

Conflict of Interest

The authors declare no conflict of interest.

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