

Research Article

The Low Level of Serum 1,25-Dihydroxyvitamin D3 and Calcium in Preeclampsia Women and Its Impact on Maternal Outcomes

Kadar Serum 1,25-Dihidroksivitamin D3 dan Kalsium yang Rendah pada Perempuan Preeklamsia dan Dampaknya terhadap Luaran Maternal

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Abstract

Objective: To investigate the association of serum 1,25-dihydroxyvitamin D3 (1,25[OH]2D3) and calcium levels in pregnancy with the risk of preeclampsia and its impact on maternal outcomes.

Methods: This cross-sectional observational study was conducted in the Obstetrics and Gynecology Department at Dr. M. Djamil Padang General Hospital from May 2021 to April 2022. Patients with normal pregnancy, diagnosed with preeclampsia, and willing to sign the informed consent were included in this study. Blood serum samples from patients were collected and examined with an ELISA kit. The collected data were then statistically analyzed with univariate and bivariate analysis.

Results: The serum 1,25(OH)2D3 levels strongly correlate with maternal outcomes (systolic and diastolic blood pressure) among preeclampsia patients, with p -value = <0.0001 . The serum 1,25(OH)2D3 levels in preeclampsia were significantly lower than in normal pregnancy (88.73 ± 42.22 vs. 111.11 ± 52.49 pg/ml), with p -value = 0.033 . The serum calcium levels in preeclampsia patients were significantly lower compared to normal pregnant women's (8.67 ± 0.49 vs. 9.55 ± 0.93 mg/dL), with p -value = <0.0001 . However, there was no association between serum 1,25(OH)2D3 and serum calcium levels in pregnancy.

Conclusion: Serum levels of 1,25-dihydroxyvitamin D3 and calcium in preeclampsia were significantly lower than in normal pregnancy. Serum 1,25-dihydroxyvitamin D3 levels were also found to have a significant correlation with systolic and diastolic blood pressure in preeclamptic patients. These findings reinforce the suggestion of the importance of vitamin D and calcium supplementation during pregnancy to reduce the risk of preeclampsia and to achieve better maternal outcomes.

Keywords: 1,25-Dihydroxyvitamin D3, calcium, hypertension, pregnancy, preeclampsia.

Abstrak

Tujuan: Mengetahui hubungan antara kadar serum 1,25-dihidroksivitamin D3 (1,25[OH]2D3) dan kalsium pada kehamilan dengan risiko preeklamsia dan pengaruhnya terhadap luaran maternal

Metode: Studi observasional potong lintang ini dilakukan di Departemen Obstetri dan Ginekologi RSUP Dr. M. Djamil Padang dari Mei 2021 hingga April 2022. Pasien dengan kehamilan normal, terdiagnosis preeklamsia, dan bersedia menandatangani informed consent diinklusi dalam penelitian ini. Sampel serum darah dari pasien dikumpulkan dan diperiksa dengan ELISA-kit. Data yang terkumpul kemudian dianalisis secara statistik dengan analisis univariat dan bivariat.

Hasil: Kadar serum 1,25(OH)2D3 memiliki hubungan yang kuat dengan luaran maternal (tekanan darah sistolik dan diastolik) pada pasien preeklamsia, dengan nilai p = $<0,0001$. Kadar serum 1,25(OH)2D3 pada preeklamsia lebih rendah secara signifikan dibandingkan kehamilan normal ($88,73 \pm 42,22$ vs $111,11 \pm 52,49$ pg/ml), dengan p -value = $0,033$. Kadar kalsium serum pada pasien preeklamsia juga secara signifikan lebih rendah dibandingkan ibu hamil normal ($8,67 \pm 0,49$ vs $9,55 \pm 0,93$ mg/dL), dengan p -value = $<0,0001$. Namun, tidak ada hubungan antara kadar serum 1,25(OH)2D3 serum dengan kadar serum kalsium pada kehamilan.

Kesimpulan: Kadar serum 1,25-dihidroksivitamin D3 dan kalsium pada preeklamsia lebih rendah secara signifikan dibandingkan kehamilan normal. Kadar serum 1,25-dihidroksivitamin D3 juga ditemukan memiliki korelasi yang signifikan dengan tekanan darah sistolik dan diastolik pada pasien preeklamsia. Temuan ini memperkuat saran akan pentingnya suplementasi vitamin D dan kalsium selama kehamilan untuk menurunkan risiko preeklamsia dan untuk mendapatkan luaran maternal yang lebih baik.

Kata kunci: 1,25-Dihydroxyvitamin D3, kalsium, hipertensi, kehamilan, preeklamsia.

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INTRODUCTION

The maternal mortality rate (MMR) is an important indicator of a nation's health status. However, the MMR in Indonesia is still remarkably high, which is 305 per 100,000 live births, the second rank after Laos among ASEAN countries.¹ The majority of maternal mortality cases are preventable, which are mostly caused by post-partum hemorrhage, infection, preeclampsia or eclampsia, prolonged or delayed parturition, and unsafe abortion.²

Preeclampsia (PE) is one of the direct causes of maternal death with prevalence around 5-8% globally. Pregnant women with PE are characterized by elevated blood pressure (systolic blood pressure \geq 140 mmHg or diastolic blood pressure \geq 90 mmHg) and proteinuria ($>$ 300 mg/24 hours) after 20 weeks gestation, with maternal organ dysfunctions. If untreated, this condition can cause further complications including eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), liver abnormality, retinal detachment, and diffuse intravascular coagulopathy.³ PE will not only negatively affect maternal health but also impact fetal growth and development, which correlates with higher fetal morbidity and mortality.⁴

PE is a multifactorial pathological condition that occurs as an accumulation of maternal, placental, and fetal factors.⁵ Many studies have shown that low levels of vitamin D links to the development of PE. This is related to the function of vitamin D in providing an immunomodulatory effects and regulating blood pressure through the renin-angiotensin system (RAS).⁶ Sunlight is the main source of vitamin D. UVB rays from the sun are absorbed by the skin and convert 7-Dehydrocholesterol into Previtamin D₃, which is then spontaneously converted into vitamin D₃ (Cholecalciferol). This vitamin D enters the blood circulation and is hydrolyzed in the liver into 25-Hydroxyvitamin D (25[OH]D). This inactive metabolite is then activated in the kidneys to 1.25-Dihydroxyvitamin D (1.25[OH]₂D).⁷ Vitamin D is brought from mother to fetus through an active form of 1.25(OH)₂D. Damage to the blood vessel cells in the placenta and kidney causes failure of 1.25(OH)₂D synthesis and impact on blood pressure regulations, causing an increase in blood pressure. This stage is the clinical stage of the etiopathogenesis of PE.⁸

1.25(OH)₂D inhibits the production of pro-inflammatory cytokines in the human placenta.

As seen in the placenta of preeclamptic women, 1.25(OH)₂D reduced the secretion of pro-inflammatory cytokines, tumor necrosis factor- α , and interleukin-6. 1.25(OH)₂D can also interfere placental Th1 cytokine production, which is increased in PE.⁹ In addition, plasma 1.25(OH)₂D and plasma renin activity were found to be inversely correlated. The RAS is essential in controlling blood pressure and is involved in the hemodynamic dysregulation of PE. In PE, plasma levels of active renin which stimulate signaling to increase systemic blood pressure, are higher than in normotensive women. 1.25(OH)₂D can decrease renin gene transcription through a vitamin D receptor-dependent process (VDR).^{6,10}

Hypertension during pregnancy is not only influenced by a deficiency of vitamin D but can also be affected by low calcium levels, which can trigger the release of renin or parathyroid hormone (PTH). This release, in turn, leads to an increase in intracellular calcium levels in vascular smooth muscle.¹¹ Previous studies have shown that serum concentration of 1.25(OH)₂D in pregnancy was positively correlated with calcium uptake.⁸ Sixty percent of patients with preeclampsia have hypocalcemia.¹² In addition, a meta-analysis of 10 studies also found that calcium supplementation was associated with a significant reduction in the incidence of preeclampsia.¹³ In contrast, a study found no significant difference in serum calcium levels between the preeclampsia and normal pregnancy groups.¹⁴

To evaluate the risk of PE based on vitamin D status, most studies examined 25(OH)D as a biomarker of vitamin D. Whereas 1,25(OH)₂D levels also have significant contributions to the pathophysiology of PE, and only limited studies focus on this active metabolite. There are also different results of the relationship of calcium levels to the occurrence of preeclampsia, while it is also essential to the development of PE. Therefore, we are interested in investigating the association of 1.25(OH)₂D₃ and calcium levels in pregnancy with the risk of preeclampsia and its impact on maternal outcomes.

METHODS

This cross-sectional observational study examined the association between vitamin D and calcium levels in pregnant women with preeclampsia and normal pregnancy. This study was carried out in the Obstetrics and Gynecology Department at Dr. M. Djamil Padang General

Hospital from May 2021 to April 2022. The inclusion criteria were pregnant women with normal pregnancy and diagnosed with PE, willing to follow the study, and signing an informed consent sheet. Malabsorption, concurrent PE, thyroid, renal, and hepatic abnormalities; ineligible serum owing to damage; and dropped individuals were the exclusion criteria. Samples were collected by consecutive sampling. Blood serum samples (5mL) from patients were collected and examined with an ELISA kit (R&D system).

The data were initially processed with univariate analysis to evaluate the subjects' characteristics, including age, parity, BMI, gestational age, and maternal outcomes (systolic and diastolic blood pressure, mean arterial pressure, serum levels of 1.25-Dihydroxyvitamin D3 and calcium). Then, the subsequent bivariate analysis was performed to analyze the significance of 1.25-Dihydroxyvitamin D3 and calcium levels between normal pregnancy and PE. Data normality was assessed based on the Shapiro-Wilk test. All p values < 0.05 was considered statistically significant. This study has been approved by the Health Research Ethics Committee of Andalas University (approval

number: 339/KEPK/2021).

RESULTS

A total of 80 pregnant women were observed in this study, 40 women with normal pregnancy and 40 women with preeclampsia.

Table 1 shows the characteristics of patients in this study. The mean age of patients with normal pregnancy was older than the PE. Patients with PE had a higher BMI than normal pregnant. In both groups, the number of patients with multiparity was higher than those who with the first pregnancy. The gestational age in patients with normal pregnancy was higher than in PE patients. The systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were found higher in the patients with PE compared to normal pregnant women. The levels of 1.25-Dihydroxyvitamin D3 and calcium in normal pregnancy were higher than in PE patients, which were 111.11 ± 52.49 vs. 88.73 ± 42.22 pg/ml and 9.55 ± 0.93 vs. 8.67 ± 0.49 mg/dL, respectively.

Table 1. Subject Characteristics

Characteristics	Normal Pregnancy (n = 40)	Preeclampsia (n = 40)
Age (years), mean \pm SD	33.13 \pm 4.99	30.48 \pm 6.82
Parity, n (%)		
Primipara	6 (15.0)	16 (40.0)
Multipara	34 (85.0)	24 (60.0)
BMI (kg/m ²), mean \pm SD	22.75 \pm 3.24	26.73 \pm 5.30
GA (weeks), mean \pm SD	37.50 \pm 1.22	32.58 \pm 5.10
SBP (mmHg), mean \pm SD	117.40 \pm 7.21	164.63 \pm 18,93
DBP (mmHg), mean \pm SD	76.00 \pm 5.06	101.30 \pm 7.67
MAP (mmHg), mean \pm SD	89.60 \pm 4.54	122.03 \pm 10.15
1,25(OH) ₂ D (pg/ml), mean \pm SD	111.11 \pm 52.49	88.73 \pm 42.22
Calcium (mg/dL), mean \pm SD	9.55 \pm 0.93	8.67 \pm 0.49

BMI=Body Mass Index; GA=Gestational Age; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MAP=Mean Arterial Pressure

Our study showed a significant difference of 1.25-Dihydroxyvitamin D3 serum value in PE and normal pregnancy (72.53 vs. 99.18 pg/ml; $p = 0.33$). Similarly, the serum calcium level of pregnant women with PE is significantly lower

than normal pregnant women (8.67 vs. 9.55 mg/dL; $p = <0.0001$), as seen in Table 2. The result of linear regression statistical test showed that there was no correlation between serum 1,25(OH)₂D₃ and serum calcium level ($r=0.167$; $p=0.302$).

Table 2. The Differences of Serum 1,25-Dihydroxyvitamin D3 and Calcium Levels between Normal Pregnancy and Preeclampsia

Variables	Normal Pregnancy (n = 40)	Preeclampsia (n = 40)	P-value
1.25-dihydroxyvitamin D3 (pg/ml)	99.18 (34.38-263.62)	72.53 (25.86-215.23)	0.033*
Calcium (mg/dL)	9.55 (7.70-11.80)	8.67 (7.60-10.30)	<0.0001*

*Values are presented in median (min-max)

The current study also evaluated the difference of 1.25-dihydroxyvitamin D3 regarding maternal outcomes in pregnant women with PE. We found that there was a significant difference in the levels

of 1.25-dihydroxyvitamin D3 based on systolic blood pressure, diastolic blood pressure, and gestational age, but not in term of body mass index. The details are provided in Table 3.

Table 3. The Differences of Serum 1.25-Dihydroxyvitamin D3 Levels based on Maternal Outcomes among Patients with Preeclampsia

Maternal Outcomes	1,25-Dihydroxyvitamin D3 (pg/ml) median (min – max)	P-value
Systolic Blood Pressure		<0.0001*
Hypertension (n = 15)	127.40 (59.19-215.23)	
Severe hypertension (n = 25)	66.62 (25.86-168.80)	
Diastolic Blood Pressure		<0.0001*
Hypertension (n = 30)	86.28 (57.05-215.23)	
Severe hypertension (n = 10)	52.80 (25.86-76.12)	
Gestational Age		0.026*
Early onset (n = 19)	67.43 (25.86-168.80)	
Late onset (n = 21)	86.38 (52.40-215.23)	
Body Mass Index		0.586
Normal weight (n = 16)	67.97 (41.73-168.80)	
Overweight (n = 16)	87.56 (25.86-215.23)	
Obesity (n = 8)	71.37 (52.40-133.92)	

DISCUSSIONS

Age, parity, and body mass index (BMI) are the risk factors for the occurrence of preeclampsia. Our study showed that PE patients were younger than the normal pregnancy. A previous study also found a relationship between maternal age and preeclampsia, in which mothers under 20 were at higher risk for preeclampsia. The reason was linked to normal trophoblast cell invasion failure, which might result in spiral arterioles not adapting correctly.¹⁵

This study also showed that 60% of pregnant women with PE are multiparous. However, this finding is different with the study result that reported there was an association of parity and the occurrence of severe preeclampsia, which primigravida mothers have 1.6 times higher risk than mothers with multigravida.¹⁶

The BMI indicates the individual's nutritional status to determine normal, underweight, overweight, or obese. In Asia-Pacific, a BMI ≥ 25 kg/m² is categorized as obese. Obesity is one of the risk factors for PE. Our study reported that the BMI of normal pregnant was lower than that of PE patients, but there was no significant association ($p > 0.05$). This finding is different to the study result that BMI has a strong correlation to vitamin D levels in PE patients.¹⁷

A study reported that PE commonly occurs in the late-onset (GA ≥ 34 weeks) than in the early onset (GA < 34 weeks).¹⁸ However, our

study different results, indicating that PE was more frequently present in early onset cases (32 weeks) compared to late onset cases (38 weeks). Furthermore, we identified a significant association between gestational age and serum 1.25(OH)₂D levels in PE patients. PE can occur both in early and late-onset pregnancy. Mothers with a history of chronic hypertension were at higher risk for early-onset PE. Additionally, maternal with a family history of chronic hypertension were higher for late-onset PE.¹⁹

The current study found that in normal pregnancy had lower blood pressure (SBP, DBP, and MAP) compared to PE patients. The SBP difference between in normal pregnant women and PE patients was also shown in the previous study, with the recurrence of PE being higher 1.94 times than the normal pregnancy.²⁰ In normal pregnancy, the RAS activation is upregulated to increase the secretion of renin, angiotensin I, and II into circulation. In PE, the levels of renin, angiotensin I, and II circulations were lower than the normal pregnancy. However, the plasma receptor for renin and the autoantibody towards angiotensin II were activated to stimuli signaling.²¹

This study also reported that 1.25(OH)₂D serum level was associated with systolic and diastolic blood pressure ($p < 0.0001$) in PE patients. Vitamin D, including its active form 1,25(OH)₂D, is known to have various effects on blood pressure regulation. It can affect the renin-angiotensin-aldosterone system (RAAS), which

plays a central role in blood pressure regulation. Vitamin D may also have anti-inflammatory and vasodilatory effects that could influence blood pressure. Some studies have investigated the relationship between vitamin D levels, including 1,25(OH)₂D, and blood pressure in women with preeclampsia. Sasan et al. stated that sufficient vitamin D levels can lower arterial blood pressure.²⁰ A supplementation of >100 nmol/L 25(OH)D can lower SBP and DBP as much as -7.5 mmHg and -4,4 mmHg, respectively.²²

In this study, we reported that the serum levels of 1.25 dihydroxy vitamin D in PE patients were lower than in normal pregnancy. Meanwhile, the need for vitamin D during pregnancy is higher as it is necessary for the mother's health and the fetus's development. In PE, poor control of effector T cells by regulatory T-sets can lead to altered placental invasion, resulting in the release of vasoconstrictive factors by the placenta and consequently causing maternal hypertension and proteinuria. 1.25(OH)₂D, through its immunomodulatory role, helps maintain immune homeostasis and prevents placental vasoconstriction, so the risk of preeclampsia can be reduced.²³

A relationship between vitamin D and PE and the association with maternal blood pressure. An increase of vitamin D 30 nmol/L will reduce the risk for PE during pregnancy.²⁴ This is because sufficient vitamin D will maintain gene transcription of renin and lower the plasma receptor activation towards angiotensin II, lowering blood pressure.²¹ Furthermore, a study also showed that the lower serum vitamin D would increase the risk for high blood pressure. The study also reported that 1,25-dihydroxyvitamin D could suppress the increase of blood pressure by modulating blood vessel protection and function.²⁵ A meta-analysis of 27 randomized clinical trials concluded that Vitamin D administration in pregnancy was significantly associated with a reduced risk of preeclampsia. The higher dose of vitamin D supplementation, the less risk of PE. This result suggests that vitamin D supplementation can be a practical preventive strategy for the incidence of PE in pregnancy.²⁶

Not only it affects the RAS, but vitamin D also causes hypertension by taking a role in calcium homeostasis. It stimulates the generation of calcium transporters, increases calcium reabsorption in the kidney, and induces osteoclastic calcium release in bones. To compensate for this lower Ca²⁺ concentration

in the plasma caused by a vitamin D deficit, the main cells in the parathyroid gland secrete more parathyroid hormone (PTH) that can further cause elevated blood pressure.²⁷

This study shows a higher calcium level in normal pregnancy than in PE patients. A study by Dhungana et al. also reported a similar finding, which serum calcium level was lower in preeclampsia (8.10 ± 0.56 mg/dl) than control (9.59 ± 0.62 mg/dl) with p < 0.001.²⁸ A strong association between calcium levels in both normal pregnancy and PE, in which the calcium levels in pregnant women with PE were significantly lower.²⁹ Calcium is one of the most abundant substances in human body to support various physiologic functions, such as growth and development, neuronal excitability process, neurotransmitter releasing, muscle contraction, membrane integrity, and blood clotting.³⁰ During pregnancy, several physiological changes occur to maintain maternal homeostasis, while also maintain and support the growth and development for the fetus.³¹

Low calcium levels contribute to the progression of preeclampsia (PE) or hypertension during pregnancy. Decreased calcium levels lead to elevated parathyroid and renin hormone release, ultimately increasing intracellular calcium in the blood. High intracellular calcium in vascular smooth muscle results in increased vascular resistance and vasoconstriction, leading to high blood pressure. During pregnancy, factors such as hemodilution, increased urinary excretion, and calcium mineral transfer from the maternal to fetal circulation progressively reduce calcium concentration.³⁰ However, in the second and third trimesters, calcium metabolism and absorption are heightened. Sufficient calcium levels during this period reduce uterine smooth muscle contractions and help prevent delivery complications and premature birth.^{30,31} A meta-analysis conducted by the Cochrane Pregnancy and Childbirth Group reported that pregnant women with poor calcium intake can reduce their risk of preeclampsia and premature delivery by receiving high-dose calcium supplementation (1 g/day).³²

CONCLUSIONS

This study found that pregnant women with preeclampsia were typically younger, multiparous, had higher BMI, and experienced early-onset preeclampsia. Additionally, the

study revealed a strong correlation between serum 1,25-dihydroxyvitamin D3 levels and maternal outcomes, including systolic and diastolic blood pressure, among preeclampsia patients. Moreover, the serum levels of both 1,25-dihydroxyvitamin D3 and calcium were significantly lower in preeclampsia compared to normal pregnancies. However, no association was observed between serum 1.25(OH)2D3 and serum calcium levels during pregnancy. We suggest that Vitamin D supplementation need to be prioritized by the public health system as an affordable and safe method to lower the risk of PE among pregnant women, as it also have a positive impact on maternal outcomes.

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