Serum Calcium and 25-Hydroxy Vitamin D Level in Normal and Early Onset Pre-eclamptic Pregnant Women

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Serum Calcium and 25-Hydroxy Vitamin D Level in Normal and Early Onset Pre-eclamptic Pregnant Women: A Study from Indonesia

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ABSTRACT

Introduction: Pre-eclampsia is one of the most common causes of fetomaternal morbidity and mortality worldwide. This disorder is categorised into Early Onset Pre-eclampsia (EOPE) and Late-Onset Pre-eclampsia (LOPE). EOPE is usually accompanied by severe complications for both the mother and fetus, while LOPE is accompanied by relatively mild fetomaternal complications. Although the pathogenesis of EOPE is not yet fully elucidated, recent studies indicate that serum calcium and 25 Hydroxy Vitamin D (25(OH)D) levels may play a role in its pathogenesis.

Aim: To find out the relationship of calcium and 25(OH)D serum levels in pregnant women with normal pregnancy and with EOPE in Indonesia.

Materials and Methods: This study was a case-control study, conducted in Dr. Soetomo General Hospital from July to October 2017. A total of 36 women with EOPE and 64 women with normal pregnancy were included in this study. Inclusion criteria were pregnant women in 2nd or 3rd-trimester with BMI >18 kg/m^2. Blood sample analysis was done to measure serum calcium and 25(OH)D level. Data were expressed as Mean±Standard Deviation. Data distribution was analysed using Shappiro-Wilk test. Comparison of serum calcium and 25(OH)D level between groups was analysed using Independent t-test. Correlation between serum calcium and 25(OH)D level was analysed using Pearson's correlation test. The p-value of <0.05 was considered significant.

Results: There was a significant difference in serum calcium level between case and control group (8.294±0.725 vs 8.670±0.405 mg/dL; p=0.006). In 25(OH)D level, there was no difference between both groups (16.128±7.5463 vs 17.325±6.4992 ng/mL; p=0.406). No correlation was found between calcium and 25(OH)D level (r=0.165; p=0.101).

Conclusion: Calcium deficiency plays a role in the incidence of EOPE among pregnant women in Indonesia. The actual role of calcium deficiency in EOPE needs further investigation.

Keywords: Calcium deficiency, Early-Onset Preeclampsia, Fetomaternal complications, Pregnant women, 25(OH)D Deficiency

INTRODUCTION

Pre-eclampsia is one of the most common causes of maternal and fetal morbidity and mortality worldwide. In 2013, the crude incidence was 2.3% globally, ranging from 1.2-4.0% across regions. In South East Asia region, the crude incidence was 2.7% [1]. Compared to developed countries, WHO has estimated the incidence of Pre-eclampsia in developing countries as seven times higher [2]. In Indonesia, Pre-eclampsia became the second largest contributor to maternal death after haemorrhage in 2013 [3]. In Dr. Soetomo General Hospital, a centre for referral hospital in eastern part of Indonesia, Pre-eclampsia was responsible for 31% of maternal deaths in 2013-2014 [4].

Pre-eclampsia is a multisystem disorder, defined as hypertension developing after 20-weeks gestation and the coexistence of one or more of the following new onset conditions: 1) proteinuria; 2) maternal organ dysfunction; and 3) uteroplacental dysfunction [5]. Based on the onset, it is categorised into EOPE and LOPE. EOPE is defined as Pre-eclampsia which occurs at <34 weeks gestation age, while LOPE is defined as Pre-eclampsia which occurs at ≥34 weeks [6]. Although the presenting features overlap, the outcome between EOPE and LOPE are different. EOPE is usually accompanied by severe complications for both the mother and fetus, while LOPE is usually accompanied by relatively mild fetomaternal complication [7]. Previous studies which compare the clinical and perinatal outcomes between EOPE and LOPE revealed that even though the incidence of LOPE are higher than EOPE, the outcomes are worse in EOPE in terms of lower gestational age delivery, lower birthweight, lower 5-minutes APGAR score, higher incidence of stillbirth, early neonatal death, and perinatal mortality [6-8].

Until now, the pathogenesis of EOPE is not yet fully elucidated. Recent theory for the pathogenesis of Pre-eclampsia is two-staged model, where poorly perfused placenta due to the failure in placental vascular remodelling (Stage 1) leads to the clinical manifestations of EOPE (Stage 2) [9]. There are several factors that have been suggested to be involved in the failure in placental vascular remodelling, which are maternal constitutional factors, genetic, behavioural, environmental and nutrition intake, modified by the physiological changes of pregnancy [9,10]. In contrary, the pathogenesis of LOPE is associated with maternal factors. Among all maternal factors that may contribute to the LOPE, pre-pregnancy Body Mass Index (BMI) seems to play a crucial role [11]. Considering that the outcomes are more severe in EOPE than in LOPE, this study focused on the factors that contribute to the incidence of EOPE.

Despite the progress in management of Pre-eclampsia, the definitive treatment for this multi-system-disorder remains delivery of the placenta. Therefore, current studies are focusing on prevention of this disorder. It has been known for years that proper nutrition prior and throughout pregnancy is important for both mother and fetus. In developing countries, where the incidence of Pre-eclampsia is higher, pregnant woman consumes diet with lesser amounts of essential minerals and vitamins [10]. Independently, calcium and vitamin D deficiency has been associated with an increased risk of EOPE [12,13].

Calcium is needed for fetal cortical bone and trabecular mineralisation [14]. In a calcium deficiency situation, there will be maternal bone loss to fulfil the requirement. When it is exacerbated by low dietary intake, maternal bone turnover will sharply increase to keep up with the fetal requirement. Calcium absorption during pregnancy is mediated by changes in maternal calciotropic hormones. Low calcium intakes during pregnancy may stimulate the secretion of PTH, one of the calciotropic hormones, increasing intracellular calcium and smooth muscle contractibility, and/or renin release from the kidney, leading to vasoconstriction and retention of sodium and fluid. These physiological changes lead to the development of EOPE [10,14]. On the other hand, vitamin D is mainly crucial for gene synthesis in the early development of placenta and the regulation of blood calcium and phosphate level [15]. Deficiency of this vitamin will induce periphery vascular modulation disturbance and loss of renin biosynthesis suppression, which results in inadequate placental growth altogether with onset of hypertension, leading to EOPE and eclampsia [16].

However, there was no study regarding the serum calcium and vitamin D level among pregnant woman in Indonesia until the write-up of the study. The aim of this study were to measure serum levels of calcium and 25(OH)D in pregnant woman in Indonesia, in order to know whether there was any significant difference in the serum levels between pregnant women with normal pregnancy and with EOPE pregnancy in Indonesia, and to find out if there was any correlation between serum calcium and vitamin D level in pregnant women. This study did not include the LOPE pregnancy because based on the previous studies, the calcium and vitamin D deficiency was related to the incidence of EOPE and not LOPE.

MATERIALS AND METHODS

The study was a case-control study conducted from June 2017 to October 2017 in Dr. Soetomo General Hospital, Surabaya, Indonesia. Subjects were pregnant women in 2nd and 3rd trimesters. History taking, physical examination, and blood sample analysis were done to evaluate serum calcium and 25(OH)D level in each subject. Blood samples were collected from ante cubital vein using a sterile needle and syringe. To obtain the serum, blood samples were allowed to clot and then centrifuged at 3000 revolutions per minute for 10 minutes. The analysis was done immediately. Serum calcium was measured using O-Cresolphthalein-Complexone (OCPC) method with MC Reagent Calcium Assay Kit (Japan Institute for the Control of Aging (JaICA); Shizuoka; Japan). Calcium and OCPC formed a chelate complex with deep red colour, and calcium concentration was determined by the absorbance at 570 nm. Serum 25(OH)D was measured using Chemiluminescent Microparticle Immunoassay (CMIA) method with ARCHITECT 25-OH Vitamin D 5P02 (Abbot 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.

Hundred pregnant women were included in the study, of whom 36 were pregnant women with EOPE pregnancy as a case group and 64 were pregnant women with normal pregnancy as control group. EOPE Pregnancy defined as new onset of hypertension (≥140 mmHg systolic or ≥90 mmHg diastolic) which occurs between 20-34 weeks gestation, with one or more of the following new-onset conditions: 1) Proteinuria; 2) maternal organs dysfunction; 3) uteroplacental dysfunction [5]. EpiInfo™ [17] was used to calculate the sample size, using method developed by Kelsey JL et al., [18]. Inclusion criteria for case group were pregnant women aged 18-35 with BMI >18 kg/m^2, fulfilling International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria of PE [5], singleton pregnancy, and signed the informed consent. Inclusion criteria for control group were pregnant women aged 18-35 with BMI >18 kg/m^2, did not met the ISSHP criteria of PE [5], singleton pregnancy, and signed the informed consent. Pregnant woman diagnosed with chronic hypertension with/without proteinuria, pre-gestational diabetes mellitus, gestational diabetes mellitus, acute kidney injury, chronic kidney disease, methamphetamine user, and have documented autoimmune disease were excluded from this study.

This study follows the principles of the Declaration of Helsinki. This study had received ethical clearance from Dr. Soetomo General Hospital before the study began (Ethical Clearance Number 505/panke.KKE/VIII/2017). All subjects gave their informed consent prior to their inclusion in the study. Information for informed consent was given before subjects signed the informed consent. Details that might disclose the identity of the subjects under study were omitted.

Serum calcium level was presented in mg/dL, while serum 25(OH)D level was presented in ng/mL.

STATISTICAL ANALYSIS

Acquired data was analysed using SPSS version 16.0. Data were expressed as Mean±Standard Deviation. Data distribution was analysed using Shappiro-Wilk test. Comparison of serum calcium and 25(OH)D level between two groups was analysed using Independent t-test. Correlation between serum calcium and 25(OH)D level was analysed using Pearson's correlation test. The p-value of < 0.05 was considered as statistically significant.

RESULTS

From June 2017 to October 2017, 36 pregnant women with EOPE and 64 pregnant women without EOPE in Dr. Soetomo General Hospital were involved in this study. Baseline characteristic showed that the maternal ages and BMI between both groups had significant differences (p=0.027, p=0.016, respectively). Systolic, diastolic, and mean arterial pressure, all showed a statistically significant difference between both groups (p=0.0001) [Table/Fig-1].

In this study, mean serum calcium level of pregnant women with EOPE pregnancy was 8.294±0.725 mg/dL, while in pregnant women with normal pregnancy was 8.670±0.405 mg/dL. There was a significant difference between both groups (p=0.006; 95% CI=0.1124-0.6393). The mean serum 25(OH)D level of pregnant women in EOPE group was 16.128±7.5463 ng/mL, while in control group was 17.325±6.4992 ng/mL. No significant difference was found between both groups (p=0.406; 95% CI=-0.1659-4.0463) [Table/Fig-2]. Pearson's correlation test showed that there was no correlation between serum calcium and serum 25(OH)D level (r=0.165; p=0.101).

DISCUSSION

High BMI, old age, and high blood pressure are a risk factor for Pre-eclampsia. In the present study, pregnant women with EOPE pregnancy had a significantly higher BMI, older age, and higher blood pressure compared to pregnant women with normal pregnancy. This finding was in accordance with previous study, where there was a significant difference in BMI, age, and blood pressure between normal pregnancy and preeclamptic pregnancy [10]. The odds ratio for pregnant women in low- and middle-income countries with BMI between 26 and 35 in developing Pre-eclampsia is 1.71 and rising to 3.90 in pregnant women with BMI of >35 kg/m^2 [19]. Women aged more than 29-year-old have the odd ratio of 1.40 in developing Pre-eclampsia [19].

Authors found a lower calcium levels in the EOPE group. This finding was consistent with previous studies that show lower blood calcium level in pregnant women with Pre-eclampsia. Previous study from India shows that the calcium level difference is noteworthy (7.84±0.87 mg/dL Vs. 8.97±0.69 mg/dL; p<0.001) [10], and another study from Ghana also supports that calcium level in pregnant women with Pre-eclampsia was below those without Pre-eclampsia (1.168 Vs. 2.368 mmol/L; p<0.0001) [20].

	Study group (%) n=36	Control group (%) n=64	p-value
Age (Mean±SD)	30±4.951	27.78±4.638	0.027*
Parity			
0	8 (22.2)	21 (32.8)	0.466
1	15 (41.7)	24 (37.5)	
2	9 (25)	12 (18.8)	
3	2 (5.6)	3 (4.7)	
4	1 (2.8)	3 (4.7)	
5	1 (2.8)	1 (1.6)	
Gravida			
1	8 (22.2)	20 (31.2)	0.231
2	10 (27.8)	22 (34.4)	
3	12 (33.3)	12 (18.8)	
4	2 (5.6)	2 (3.1)	
5	2 (5.6)	6 (9.4)	
6	0 (0)	1 (1.6)	
7	0 (0)	1 (1.6)	
8	1 (2.8)	0 (0)	
9	1 (2.8)	0 (0)	
Body Mass Index (Kg/m^2)			
<20	0 (0)	7 (10.9)	0.016*
20-25	12 (33.3)	19 (29.7)	
25-30	8 (22.2)	27 (42.2)	
30-35	13 (36.1)	7 (10.9)	
>35	3 (8.3)	4 (6.2)	
Systolic blood pressure (mmHg) (Mean±SD)	157.08±19.434	114.38±9.074	0.0001*
Diastolic blood pressure (mmHg) (Mean±SD)	95.28±9.706	73.92±6.778	0.0001*
Mean Arterial Pressure			
<90 mmHg	0 (0)	40 (62.5)	
>90 mmHg	36 (100)	24 (37.5)	

[Table/Fig-1]: Clinical characteristic.
*p<0.05 was considered statistically significant

Variables	Pregnant women with early onset PE n=36	Normal pregnant women n=64	p-value	95% CI
Serum calcium level (mg/dL)	8.294±0.725	8.670±0.405	0.006*	0.1124-0.6393
Serum 25(OH) D level (ng/mL)	16.128±7.5463	17.325±6.4992	0.406	-0.1659-4.0463

[Table/Fig-2]: Serum calcium and 25(OH)D level in normal pregnant woman and pregnant woman with early-onset PE.
*p<0.05 was considered statistically significant

During pregnancy, especially in 2nd and 3rd-trimester, calcium requirement increases. Previous study reported that woman with a daily average calcium intake of 1171 mg during pregnancy absorbs 57% more during the second trimester and 72% during third trimester [21]. However, calcium consumption level of pregnant women in Indonesia is only 45-78% below the required estimated dose [22]. Although absorption of calcium increases during pregnancy, the supplementation of calcium is not necessary as long as daily requirement of 1000-1200 mg is fulfilled [14,23]. Hence, calcium supplementation in pregnant woman with low calcium intake can decrease intracellular calcium concentration, induce vasodilatation by decreasing blood vessels smooth muscle contraction, which will lead to decrease in blood pressure [24,25]. WHO recommends calcium supplementation of 1.5-2 gram of elemental calcium in every pregnant woman in low calcium intake area every day [13]. Meta-analysis studies show that calcium supplementation significantly decreases the prevalence of Pre-eclampsia [24,25].

In this study, no significant difference of 25(OH)D level was found between groups. This finding was in accordance with previous study which showed that neither vitamin D insufficiency (aOR=1.1; 95% CI = 0.6-2.0) nor deficiency (aOR = 1.4; 95% CI = 0.7-3.0) was significantly associated with Pre-eclampsia [26]. It is because serum 25(OH)D levels do not change during pregnancy, but an increase in 1-α hydroxylase and additional synthesis in the placenta allows for an increase in the conversion of 25(OH)D to 1,25(OH)2D. Maternal serum 1,25(OH)D levels increase 2-folds during pregnancy, allowing the intestinal absorption of calcium to double [21]. In contrary, study by Bodnar LM et al., revealed that maternal 25(OH)D concentration who develop EOPE are significantly lower than control group (45.4 nmol/L Vs. 53.1 nmol/L, p<0.01) [27]. The difference might be because in the present study, mean 25(OH)D level in both pregnant women with EOPE pregnancy and normal pregnancy were in the deficiency range (16.128±7.5463 ng/mL and 17.325±6.4992 ng/mL, respectively), whereas the normal range is 32-100 ng/mL [28].

In Southeast Asia, the prevalence of vitamin D deficiency varies from 6-70% with cut-off value of 50 nmol/L, whereas in Indonesia such specific data is still not available [29]. Pregnant women in this category are at risk of suffering bone loss and having neonates with hypocalcaemia or rickets [28]. Furthermore, early-pregnancy maternal 25(OH)D concentration <15.2 ng/L (37.5 nmol/L) has 5-fold increase in the risk of suffering EOPE despite supplementations of vitamins [27]. Another study suggests that as vitamin D relies on sun exposure, diet, and supplementation, there will only be little or no change in vitamin D level during pregnancy [30].

In this study, there was no correlation between serum calcium and 25(OH)D level. This finding was inconsistent with previous study that compare calcium and vitamin D level between calcium supplementation with vitamin D supplementation and calcium supplementation with 30 minutes of morning sun exposure. It was revealed that the level of both calcium and vitamin D was increased in group with vitamin D supplementation, but the group with morning sun exposure showed decrease level of calcium without any change of vitamin D level. [31]. Other study shows remarkable increase of 25(OH)D level in supplementation of both calcium and vitamin D compared to another group with mono-supplementation of calcium, despite no elevation of blood calcium level in both groups [32]. Another study suggests that serum 1,25(OH)2D would rise in women with low calcium intakes (<500 mg/day) and those with adequate intakes in late pregnancy, though to a greater or lesser extent depending upon intake [14,21]. However, both groups in this study shows low level of vitamin D. Authors suggest that due to the low level of both serum Calcium and 25(OH)D in this study, there was no feedback mechanism that was crucial in calcium metabolism as explained below.

In normal condition, calcium is maintained between 8.5 mg/dL–10.5 mg/dL in the blood through the interaction of Parathyroid Hormone (PTH), 25(OH)D, and 1,25(OH)2D. In deficiency state, secreted PTH will stimulate kidney to convert 25(OH)D into its active form, 1,25(OH)2D to reabsorbs calcium from the bone, intestines, and kidneys [23,33]. Calcium is then transported using either the active transport system, which consumes the Adenosine Triphosphate (ATP), or passive transport system through diffusion, which depends on gradient i.e., through calcium intake. However, in vitamin D insufficiency, converted 1,25(OH)2D is inadequate to balance blood calcium level, thus diffusion predominates calcium transport system [34,35]. Low calcium intake would also aggravate such condition since it demands a higher production of 1,25(OH)2D and breakdown of its metabolites. Thus, adequate vitamin D status allows calcium intake among individuals to become more flexible [35].

LIMITATION

The sample size was low due to the limited funding that the authors received. There was a wide variability of the gestational age of pregnant women within the inclusion criteria, ranging from 2nd to 3rd-trimester.

CONCLUSION

Serum calcium level in pregnant women with EOPE pregnancy are significantly lower compared to pregnant women with normal pregnancy. Serum 25(OH)D level in pregnant women with EOPE pregnancy are not significantly lower compare to pregnant women with normal pregnancy. There is no correlation between serum calcium and 25(OH)D level. It showed that calcium deficiency plays a role in the incidence of EOPE among pregnant women in Indonesia. The actual role of calcium deficiency in EOPE needs further investigation.

Authors' Contribution

MIAA and FFA are equally contributed as the primary authors in the conception, methodology, supervision, and preparation of the initial and final manuscript. AAHH contributed to the methodology, formal analysis, and review of the manuscript. DKI contributed to the fund acquisition and project management. YK contributed to conception and methodology. RAN contributed to the formal analysis and review of the manuscript. TNO and MJ contributed to the data collection and provided sources.

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