ABSTRAK
Preeklamsia pada ibu hamil merupakan salah satu penyebab morbiditas dan mortalitas maternal dan perinatal baik di dunia maupun di Indonesia. Kadar kalsium darah yang rendah pada ibu hamil diduga berperan terhadap patogenesis preeklamsia. Penelitian ini bertujuan untuk mengetahui perbedaan kadar kalsium darah pada preeklamsia dengan kehamilan normotensi di RSUD Prof. Dr. Margono Soekarjo Purwokerto. Studi analitik observasional dengan pendekatan case control pada tiga kelompok penelitian yaitu preeklamsia ringan (PER), preeklamsia berat (PEB), dan kehamilan normotensi sebagai kelompok kontrol. Jumlah subjek tiap kelompok berjumlah 21 orang. Sampel darah diambil dari vena antecubiti dan disentrifuge untuk diambil serumnya. Pengukuran kadar kalsium dilakukan dengan metode Chresol Pthalein Complex. Hasil pada penelitian ini didapatkan rerata kadar kalsium darah pada kelompok PEB paling rendah (8,34±0,32mg/dL) dan berbeda bermakna secara statistik dibandingkan kelompok PER (8,59±0,33mg/dL) dan normotensi (8,71±0,31mg/dL). Penelitian ini membuktikan potensi keterlibatan kalsium pada preeklamsia berat.

Kata Kunci: Kalsium darah, kehamilan, preeklamsia

ABSTRACT
Preeclampsia in pregnant women is one of the causes of maternal and perinatal morbidities and mortalities both in the world and in Indonesia. Low blood calcium levels in pregnant women are thought to contribute to preeclampsia pathogenesis. This study aimed to determine the differences of blood calcium level in preeclampsia with normotensive pregnancy at RSUD Prof. Dr. Margono Soekarjo Purwokerto. This was an observational analytic study with case control approach which consisted of three groups, i.e. mild preeclampsia, severe preeclampsia, and normotensive pregnancy as the control group. The number of subjects per group was 21 people. Blood samples were taken from the antecubital vein and centrifuged for serum. Measurement of calcium level was done by Chresol Pthalein Complex method. The results of this study showed that the lowest average blood calcium level was in severe preeclampsia group (8.34±0.32mg/dL) and statistically different significantly compared to those in mild preeclampsia group (8.59±0.33mg/dL) and normotensive group (8.71±0.31mg/dL). This study proves the potential of calcium involvement in severe preeclampsia.

Keywords: Blood calcium, preeclampsia, pregnancy

Corresponding Author: Ika Murti H. Bagian Histologi Fakultas Kedokteran Universitas Jenderal Soedirman Purwokerto, Jl. Dr. Gumbreg No. 1 Purwokerto Tel. (0281) 622022 Email: murti_cool@yahoo.com

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INTRODUCTION

Preeclampsia is a hypertensive disease in pregnancy characterized by hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90mmHg) accompanied by proteinuria (≥300 mg/dL in 24-hour urine collection) at gestational age more than 20 weeks or immediately after delivery (1). Preeclampsia is one of the main causes of maternal and perinatal morbidity and mortality both in the world and in Indonesia in addition to bleeding and infection or sepsis (2). The incidence of preeclampsia around the world ranges from 2-10% of pregnancies, and WHO estimates the incidence of preeclampsia is seven times higher in developing countries than in developed countries (3). The incidence of preeclampsia in Indonesia is 128,273 per year or around 5.3% (4). Preeclampsia is a serious health problem since it has an impact on infants including prematurity, low birth weight, inhibited fetal growth which contributes to the number of perinatal morbidity and mortality (4).

The mechanism or pathogenesis of preeclampsia is quite complex. At present, the theory of the preeclampsia mechanism can be divided into two stages. The first stage is asymptomatic, without signs and symptoms. In this first stage, there is an abnormal placental formation and placental factor release in the maternal circulation. The second stage is symptomatic, that is characterized by hypertension and proteinuria. In severe conditions, brain angiospasm can cause seizures (5).

Another factor that is thought to play a role in the occurrence of preeclampsia is calcium. Low blood calcium level is a factor in the development of hypertension in pregnancy and preeclampsia. During pregnancy, calcium concentration decreases progressively because of the hemodilution process, increased urine secretion, increased transfer of calcium minerals from mother to fetus, and low calcium intake in pregnant women (6). However, the role of calcium in preeclampsia is still controversial. Some studies reported a lower blood calcium level in preeclampsia than in the control or normotensive group (6,7,8,9). Meanwhile, other studies reported that there were no differences in calcium levels between the preeclampsia and normotensive pregnancy groups (10).

Prof. Dr. Margono Soekarjo Regional General Hospital Purwokerto is a referral hospital in the southern and western province of Central Java that receives many pregnancies with mild preeclampsia, severe preeclampsia, and normotensive pregnancy at Prof. Dr. Margono Soekarjo Regional General Hospital.

The sampling technique used was a non-probability sampling method. Sample was selected using consecutive sampling, i.e., each patient who came in sequence and fulfilled the sample criteria would be selected until the research quota was met. In this study, calcium levels were examined among three groups, namely the mild preeclampsia group, the severe preeclampsia group, and the normotensive pregnancy group as the control group at gestational age ≥20 weeks at Prof. Dr. Margono Soekarjo Regional General Hospital during the study period with the same number of sample in each group. Based on the sample calculation for a case-control study (11), the sample size for each group was 19. The number of samples was increased by 10% into 21 people in each group to anticipate the possibility of dropping out.

Obstetrics and Gynecology specialist doctors determined the preeclampsia diagnosis under the following criteria: (a) Mild preeclampsia, if systolic blood pressure was ≥140mmHg or diastolic pressure was ≥90mmHg accompanied by protein excretion in urine with a level of 300mg/dL in a 24-hour urine collection or with a qualitative examination ≥1+ in random urine sampling; (b) Severe preeclampsia, if systolic blood pressure was ≥160mmHg or diastolic blood pressure was ≥110mmHg with protein excretion in urine with a level of 300mg/dL in a 24-hour urine collection or with a qualitative examination ≥1+ in random urine sampling, followed by one of these criteria: (1) platelets <100,000 per mm³, (2) increase in liver enzymes ≥2x than normal, (3) renal insufficiency (creatinine serum > 1.1 mg/dl) or oliguria (output urine <500 ml in 24 hours), (4) pulmonary edema or cyanosis, (5) new cerebral or onset visual disturbances, (6) persistent severe pain in the right upper quadrant or epigastric region. Subjects in the control group or normotensive pregnancy were determined under the criteria of a pregnancy condition with systolic blood pressure of 100-140 mmHg and diastolic blood pressure of 60-90 mmHg without proteinuria. Blood samples from respondents were taken through antecubital vein as much as 3cc. Blood was centrifuged, the serum was taken, and then calcium level was measured by using the Creso Phthalain Complex (CPC) method at the Clinical Pathology Laboratory of the Prof. Dr. Margono Soekarjo Regional General Hospital Purwokerto. The principle of this examination is that calcium ion reacts with o-cresolphthalain complexone in an alkaline atmosphere to form a purplish complex. The complex absorbance of this color is proportional to the calcium concentration in the sample.

Data on maternal age, gestational age, and calcium level were analyzed using the ANOVA parametric test because the data types were numerical and the data distribution was normal and homogeneous. Then, the post hoc LSD test was conducted to determine the differences in calcium levels among the research groups. The results of the analysis were considered significant if p <0.05. The difference in parity status was analyzed using the chi-square test because of the nominal categorical data type (12).

METHOD

This research has gone through a study of ethics by the Health Research Ethics Commission Faculty of Medicine, Jendral Sudirman University number 1448/UN.23.07.5.1/PN.1/2017. Observational analytic design using a case-control approach was conducted to determine the differences in blood calcium levels in pregnancies with mild preeclampsia, severe preeclampsia, and normotensive pregnancy at Prof. Dr. Margono Soekarjo Regional General Hospital.
RESULTS

The results showed that the mean age of mothers with severe preeclampsia (SPE) was the highest compared to the control group (normotensive/NT) and the mild preeclampsia (MPE) group even though was not statistically significant (p = 0.545). In the SPE group, it was found that the average gestational age of mothers was not yet a term or 34.86 ± 3.76 weeks lower than the normal pregnancy and MPE groups although it was not statistically significant (p = 0.277). In this study, parity status in all groups at most was multipara. Based on statistical analysis, there were no differences in parity status among the research groups (p = 0.599) (Table 1). Both groups can be said to be homogeneous regarding maternal age, gestational age, and parity status.

Table 1. Characteristics of research subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control or NT (n=21)</th>
<th>MPE (n=21)</th>
<th>SPE (n=21)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)</td>
<td>29.65±6.30</td>
<td>31.28±6.81</td>
<td>32.09±7.63</td>
<td>0.545a</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>37.05±3.37</td>
<td>36.09±5.69</td>
<td>34.86±3.76</td>
<td>0.277a</td>
</tr>
<tr>
<td>Parity status (individual)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>8</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Primipara/multipara</td>
<td>13</td>
<td>16</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

Note: 
- p-value)a no significant differences using the ANOVA test
- p-value)b no significant differences using the Chi-Square test

In the severe preeclampsia group, maternal gestation age was the lowest compared to those in the normotensive group and MPE. This is consistent with a study that reported the gestational age of mothers with preeclampsia is lower than the gestational age of mothers in the control group (16). The study also reported that the factor influencing the occurrence of preeclampsia in addition to maternal gestational age is a history of previous preeclampsia in the mother (16). Another study reported that the mean gestational age of mothers with preeclampsia is 39±2.4 weeks (17), and it is also reported that if the mother’s gestational age is a term (>37 weeks), thus the birth weight is not different from the weight of the baby born from a mother with normotensive pregnancy.

In this study, most of the mothers who experienced preeclampsia were those who had previously given birth either primi or multipara. This is in line with research that reported mothers who have given birth have a greater chance of developing preeclampsia (15). While other report stated that nullipara or mothers who have just been pregnant and have never given birth before having a higher chance of developing preeclampsia (18). The cause of the differences in the research conducted with previous research is likely because there are other risk factors not examined in this study. Other risk factors that play a role in preeclampsia include the presence of maternal diseases such as kidney disease, antiphospholipid antibody syndrome, systemic lupus erythematosus, chronic hypertension, and diabetes mellitus. Other factors such as obesity, multiple pregnancies, and previous history of preeclampsia also play a role in preeclampsia (18). These factors were not examined in this study.

This study identified significant differences in calcium levels with the lowest calcium level in SPE, with the lowest gestational age, and most multiparous. The mean age of mothers with severe preeclampsia was 32.09±7.63 years, the highest compared to the normotensive group and MPE. Even though the mean is the highest, this age is still included in the range of normal reproduction age. Some studies suggest that the age of mothers over 35 years has a higher chance of developing preeclampsia than the age of mothers under 35 years (13,14). Meanwhile, other studies report that the higher the age of the mother (>40 years), the higher the risk of developing preeclampsia and other risks including the risk that occurs in antenatal, the risk of labor, and complications that occur in the fetus (15).

In the severe preeclampsia group, maternal gestation age was the lowest compared to those in the normotensive group and MPE. This is consistent with a study that reported the gestational age of mothers with preeclampsia is lower than the gestational age of mothers in the control group (16). The study also reported that the factor influencing the occurrence of preeclampsia in addition to maternal gestational age is a history of previous preeclampsia in the mother (16). Another study reported that the mean gestational age of mothers with preeclampsia is 39±2.4 weeks (17), and it is also reported that if the mother’s gestational age is a term (>37 weeks), thus the birth weight is not different from the weight of the baby born from a mother with normotensive pregnancy.

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This study identified significant differences in calcium levels with the lowest calcium level in SPE. The normotensive group had the highest mean blood calcium level but was not significantly different from the MPE group. Calcium level in the normotensive group was included in the normal range because it is known that normal calcium level in adult women is 8.5-10mg/dL. The lowest mean blood calcium level was found in the SPE group, which was 8.34 ± 0.32mg/dL. The results also illustrate the more severe the degree of preeclampsia, the lower the blood calcium level. This is in accordance with a study which also reported the blood calcium level in the SPE group is the lowest compared to the normotensive group and MPE (6). Likewise, other studies have reported low calcium level in pregnant women with preeclampsia compared to pregnant women who are not suffering from preeclampsia (7,21,22).

Preeclampsia has complex pathogenesis. A study reported the involvement of Human Leukocyte Antigen-C (HLA-C)
and Natural Killer cells (NK cells) in the process of preeclampsia (19). Other study reported the role of inflammatory cytokines, especially Tumor Necrosis Factor-α (TNF-α) in causing preeclampsia symptoms (20). Many studies have reported the involvement of minerals such as calcium in the pathogenesis of preeclampsia. Calcium is highly needed for normal development and maintaining body functions. Calcium is needed for various important processes such as neuron excitability, neurotransmitter release, muscle contraction, membrane integrity, and blood clotting (6). Calcium also plays an important role during pregnancy.

The role of low calcium in pregnant women in causing preeclampsia can be explained through several mechanisms, including when calcium level is low, it will increase parathyroid hormone expenditure and subsequently cause an increase in intracellular calcium in vascular smooth muscle. This increase in intracellular calcium in vascular smooth muscle will cause an increase in vascular resistance and vasoconstriction which will lead to an increase in blood pressure (6,23). Another mechanism is by stimulating the release of renin which will increase angiotensin II and will increase blood pressure. This low calcium level can also reduce blood magnesium level and subsequently trigger vascular smooth muscle vasoconstriction and will reduce the effects of endothelial nitric oxide synthase (eNOS), a calcium-dependent enzyme that inhibits blood vessel vasodilation. This vasodilation inhibition is also caused by a decrease in prostacyclin in the circulation. Prostacyclin is a calcium-dependent enzyme and potent vasodilator (24).

Low calcium level in pregnant women can be caused by hemodilution process, increased urine secretion, increased transfer of calcium minerals from mother to fetus, and low calcium intake in pregnant women (6). Metabolism or absorption of calcium in pregnant women will increase especially in the second and third trimesters (25). This absorption increase is related to calcium intake in the mother. The limitation of this study included the absence of maternal calcium intake, so it was not known whether low calcium levels were caused by low intakes or other causes. If the cause is indeed due to low calcium intake, additional calcium is needed during pregnancy with a dose of 1.5–2.0g calcium/day (26). Calcium supplementation is recommended to prevent preeclampsia, especially in women who are at risk of previous preeclampsia (19,26). Other limitations in this study are that this research was not examining other minerals that play a role in the incidence of preeclampsia such as magnesium and zinc, and this research did not examine the food intake consumed by pregnant women. The study also did not examine the other risk factors that play a role in preeclampsia such as a history of maternal disease, obesity, multiple pregnancies, and a history of previous preeclampsia.

The results of this study which shows that blood calcium levels in SPE are significantly lower than those in MPE and normotensive pregnancies supports the evidence of the potential of calcium involvement in the preeclampsia pathogenesis. Further studies need to be done by looking at calcium intake during pregnancy to assess the factors that cause low calcium levels in pregnancy.

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REFERENCE


