THE DIFFERENCES IN BLOOD LEAD LEVELS IN WOMEN WITH GESTATIONAL HYPERTENSION OR PRE-ECLAMPSIA AND WOMEN WITH NORMAL PREGNANCY (A STUDY IN THE NORTH COAST OF JAVA, BREBES DISTRICT)

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Abstract

Introduction: Gestational Hypertension (GH) and Pre-eclampsia (PE) are the leading causes of maternal death. Exposure to lead from the environment, especially agricultural activities, may cause pregnant women to suffer from GH and PE. GH/PE may occur through oxidative stress mechanisms and progressive endothelial damage. Brebes District is one of the areas with the highest Maternal Mortality Rate (MMR) in Central Java where the occurrence of GH/PE was a lot. This study aimed to analyze the differences in Blood Lead Levels (BLL) in pregnant women with GH/PE and normal pregnant women in Brebes District.

Methods: This study used a cross-sectional design. By selecting and applying inclusion criteria, 18 subjects were selected for the GH/PE group (case group), and 46 subjects were selected for the normotension group (control group). Measurement of BLL was carried out using the Atomic-Absorption Spectrometry (AAS). Some of the statistical methods used were independent t-test, Mann-Whitney test, Chi-square test, multivariate logistic regression test, and Rank-Spearman correlation test. The risk estimate was calculated from the Odds-Ratio (OR) and 95% Confidence Interval (CI).

Results and Discussion: The median value and range of the BLL in the case and control groups were 40.20 g/dL (15.50-89.20) and 32.75 g/dL (3.60-42.80), respectively (p = 0.011). Pregnant women with the BLL of ≥ 35.15 g/dL had eight times the risk of experiencing GH/PE (Adj-OR = 8.1; 95% CI = 1.7-39.0). Exposure to lead will increase the production of Reactive Oxygen Species (ROS), resulting in oxidative stress and endothelial dysfunction in addition to increasing the blood pressure.

Conclusion: The BLL in pregnant women with GH/PE is higher than women with normal pregnancy, and the BLL in the high category is a risk factor for GH/PE.

INTRODUCTION

Maternal death occurs during pregnancy and childbirth or within 42 days of termination of pregnancy. It is still a health problem worldwide, especially in developing countries. Every day, around 810 women die during pregnancy or childbirth (1). In addition to bleeding and infection after delivery, the leading cause of maternal death is an increase in blood pressure after 20 weeks of gestation, known as Gestational Hypertension (GH) or Pre-eclampsia (PE) (2-3). GH is a systolic blood pressure (SBP) of at least 140 mmHg and/or a Diastolic Blood Pressure (DBP) of at least 90 mmHg after the 20th week of pregnancy. If the increase in blood pressure is accompanied by proteinuria, it is referred to PE (4). The data show GH/PE accounts for about 18% of maternal deaths globally or around 62,000 to 77,000 deaths per year (4-5).

The pathophysiology of GH/PE is still unclear, but disturbances in the process of placentation in early pregnancy followed by an inflammatory process and progressive endothelial damage are strongly suspected as the disorder (6-7). Some of the risk factors for GH/PE that have been widely discussed are primiparas, history of hypertension or impaired renal function before pregnancy, history of hypertension or GH/PE in previous pregnancies, diabetes, obesity, age over 40 years, multiple pregnancies, family history of PE, and so on (5-8). Several recent studies have shown that environmental factors have the potential to trigger the incidence of GH/PE, including exposure to toxic materials e.g., pesticides (9-10) and heavy metals, such as lead or plumbum (Pb) (11-12). An increase in Blood Lead Levels (BLL) is followed by the rise in blood pressure of pregnant women (13), through oxidative stress mechanisms. Increased Reactive Oxygen Species (ROS) (14) in the mechanism triggers endothelial dysfunction and increases blood pressure (15-16).

Data from ASEANstats showed that the Maternal Mortality Rate (MMR) in Indonesia in 2014 was still relatively high at 190/100,000 live births (17) and still
far from the Sustainable Development Goal's (SDGs) target at 70/100,000 live births by 2030 (18). The MMR in Indonesia is better than Laos and Myanmar but the rest of South East Asia regions (17). Central Java is one of the provinces that has the largest MMR in Indonesia (19), which is at 76.9/100,000 live births (20) ranked second nationally (19).

In 2019, Brebes District, located on the Northern Coast of Java, is the highest contributor to maternal mortality in Central Java, having 37 cases (20). Data from the Brebes District Health Office showed that one of the main causes of maternal mortality in this area is GH/PE at 67.7% (21). Environmental factors (pollution), one of which is exposure to lead, are likely suspected of contributing to the high rate of GH/PE incidence in Brebes District. The exposure to lead may come from industrial, transportation, and agricultural activities.

In everyday life, exposure to lead comes from sanding paint or house dust, eating and drinking utensils, cosmetics, glass pots, soil and drinking water from lead pipes, faucets, and plumbing fixtures (22). Several studies on the North Coast of Java have also proven that other potential risks of exposure include fish (23), vegetables, especially shallots (24), which are the main agricultural commodities in Brebes District. In addition to industrial activities and transportation, agriculture, and battery recycling activities in the area might be the main source of lead (25). The transmission routes of lead mainly occur as pregnant women get involve in agricultural activities and consume lead contaminated food or beverages daily. This study aimed to analyze the differences in BLL in pregnant women with GH/PE and women with normal pregnancy. Besides, it proved whether the high BLL is a risk factor for GH/PE in pregnant women.

METHODS

This study is an analytic observational study with a cross-sectional design. The target population was all pregnant women in the North Coast of Java. The accessible population was pregnant women who were registered at two primary healthcare centers in the North Coast of Java, Brebes district, in September and October 2017. In total, there were 275 people. The primary healthcare centers were chosen because they had the highest number of pregnant women among the areas of the coast. To control the confounding variables, inclusion criteria were applied for the subjects such as age of subjects at 20-35 years, the gestational age of >20 weeks, and singleton pregnancy.

The data obtained from the primary health care centers showed out of 275 pregnant women, 179 (65.1%) met the inclusion criteria, and 24 of them (13.4%) were the case (GH/PE) group for having the Systolic Blood Pressure (SBP) of 140 mmHg or more, or Diastolic Blood Pressure (DBP) of 90 mmHg or more, or both, with or without proteinuria. While out of 155 subjects with normotensive, 50 subjects were randomly selected as the control (normotensive) group. Of 24 subjects in the GH/PE group, six subjects refused to participate in the study for fear of having blood collection, while in the control group 4 subjects refused to participate for the same reason. The analysis was carried out to 18 subjects in the GH/PE group and 46 subjects in the normal group. The flow chart of the subjects selection can be seen in Figure 1.

The data on increased Blood Pressure (BP) above 140/90 mmHg after 20 weeks of gestation were taken from the medical records during the Antenatal Care (ANC) there. The medical records were done by trained health personnel (midwives). The use of medical records determined whether the subjects were in the GH/PE group or the normal pregnancy group. In carrying out the research, BP measurements were also carried out (as a cross-check and referred to current BP) by trained research members, using the Microlife digital sphygmomanometer. The measurements were repeated thrice and averaged automatically. Anthropometric measurements (height and weight) were performed using the Seca® 213 stadiometer and Seca® 813 electric flat scale.

The measurements of BLL were carried out using the Atomic-Absorption Spectrometry (AAS) method by the GAKY Laboratory, Faculty of Medicine, Universitas Diponegoro. Information about the characteristics of the subjects (age, maternal education level, maternal occupation), the number of pregnancies (gravida), family history of hypertension, and family history of Diabetes Mellitus (DM) was taken during interview using a structured questionnaire. Data were analyzed using SPSS version 20 software.

Some of the statistical methods used were independent t-test, Mann-Whitney test, Chi-square test, multivariate logistic regression test, and Rank-Spearman correlation test. The risk estimate was calculated from the Odds-Ratio (OR) and 95% Confidence Interval (CI). To determine the cut-off value of women’s ages, Body Mass Index (BMI), and BLL as predictors/risk factors for GH/PE, the Receiver Operating Characteristic (ROC) curve method was used. The Area Under Curve (AUC), sensitivity and specificity values were considered to determine the cut-off value (26). A multivariate logistic regression test using the Backward Wald method was
used to identify the main risk factors for GH/PE from the three candidate variables, i.e., maternal age, BMI, and BLL (27). The research was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro (No. 664/EC/FK-RSDK/XI/2017).

**RESULTS**

Even though the inclusion criteria for women’s age was applied (20-35 years old), it turned out that the GH/PE group was mostly older than the normal group. There was a significant difference in ages between GH/PE and normal groups (p-value < 0.001).

The data showed that the gestational age and height of the subjects were relatively the same in both groups (p = 0.970; p = 0.747, respectively). However, the BMI in the case (GH/PE) group was higher than the control (normotensive) group (p = 0.018) (Table 1). The results showed there was a significant difference in current systolic and diastolic blood pressures between the case and control groups (p < 0.001 for both). Regarding maternal education level, gravida, maternal occupation, family history of hypertension, and family history of DM, there were no significant differences between the two groups (p > 0.05 for both).

The Mann-Whitney U-test proved that the BLL in the case group was higher compared to the control group (p = 0.011). The median value and range of BLL in the case and control group were 40.20 g/dL (15.50-89.20) and 32.75 g/dL (3.60-42.80), respectively (Figure 2).

Regarding age, BMI, and BLL between the groups, the cut-off values were determined using the ROC curve (Figure 3). The results obtained a value of 31.5 years for maternal age (AUC=0.809; sensitivity = 88.9; specificity = 76.1), 28.4 kg/m² for BMI (AUC=0.708; sensitivity = 72.2; specificity = 63.0), and 35.15 µg/dL for BLL (AUC = 0.707; sensitivity = 72.2; specificity = 69.6). There were significant relationships between age, BMI, and BLL with the incidence of GH/PE (p < 0.05 for both) (Table 2).
### Table 1. Characteristics of GH/PE and Normal Groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GH/PE Group (n = 18)</th>
<th>Normal Group (n = 46)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.1 ± 1.97</td>
<td>28.4 ± 4.52</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Gestational ages (weeks)</td>
<td>25.5 (21 - 40)</td>
<td>27.0 (21 - 38)</td>
<td>0.970</td>
</tr>
<tr>
<td>Weight* (kg)</td>
<td>71.2 ± 12.09</td>
<td>63.4 ± 12.10</td>
<td>0.024*</td>
</tr>
<tr>
<td>Height* (cm)</td>
<td>153.5 ± 5.53</td>
<td>153.0 ± 5.06</td>
<td>0.747*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.1 ± 4.14</td>
<td>27.0 ± 4.67</td>
<td>0.018</td>
</tr>
<tr>
<td>Current Blood Pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>158.9 ± 16.34</td>
<td>109.1 ± 13.80</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>99.4 ± 11.40</td>
<td>68.6 ± 10.46</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Maternal education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>11 (61.1%)</td>
<td>26 (56.5%)</td>
<td>0.660</td>
</tr>
<tr>
<td>Junior high school</td>
<td>7 (38.9%)</td>
<td>18 (39.1%)</td>
<td></td>
</tr>
<tr>
<td>Senior high school</td>
<td>0 (0.0%)</td>
<td>2 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Gravida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravida</td>
<td>1 (5.6%)</td>
<td>12 (26.1%)</td>
<td>0.136*</td>
</tr>
<tr>
<td>Multigravida</td>
<td>17 (94.4%)</td>
<td>34 (73.9%)</td>
<td></td>
</tr>
<tr>
<td>Maternal Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>11 (61.1%)</td>
<td>36 (78.3%)</td>
<td>0.054</td>
</tr>
<tr>
<td>Private Employees</td>
<td>2 (11.1%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>5 (27.8%)</td>
<td>10 (21.7%)</td>
<td></td>
</tr>
<tr>
<td>Family History of Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (38.9)</td>
<td>16 (34.8)</td>
<td>0.986</td>
</tr>
<tr>
<td>No</td>
<td>11 (61.1)</td>
<td>30 (65.2)</td>
<td></td>
</tr>
<tr>
<td>Family History of Diabetes Mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (11.1)</td>
<td>6 (13.0)</td>
<td>1.000*</td>
</tr>
<tr>
<td>No</td>
<td>16 (88.9)</td>
<td>40 (87.0)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: values = mean ± SD; median (range); and frequencies, n (%).
*Independent t-test, 'Mann-Whitney test, 'Chi-square

### Figure 2. The Differences in BLL between GH/PE and Normal Group

### Figure 3. The ROC Curve for the Determination of Maternal Age, BMI, and BLL Cut-Off Values

### Table 2. The Association between Maternal Age, BMI, and BLL and the Incidence of GH/PE

<table>
<thead>
<tr>
<th>Variables</th>
<th>GH/PE Group (n = 18)</th>
<th>Normal Group (n = 46)</th>
<th>p-values</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 31.5</td>
<td>16 (88.9)</td>
<td>11 (23.9)</td>
<td>&lt;0.001</td>
<td>25.4 (5.0-128.4)</td>
</tr>
<tr>
<td>&lt; 31.5</td>
<td>2 (11.1)</td>
<td>35 (76.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 28.4</td>
<td>13 (72.2)</td>
<td>17 (37.0)</td>
<td>0.024</td>
<td>4.4 (1.3-14.6)</td>
</tr>
<tr>
<td>&lt; 28.4</td>
<td>5 (27.8)</td>
<td>29 (63.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLL (µg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 35.15</td>
<td>13 (72.2)</td>
<td>14 (30.4)</td>
<td>0.006</td>
<td>5.9 (1.8-19.9)</td>
</tr>
<tr>
<td>&lt; 35.15</td>
<td>5 (27.8)</td>
<td>32 (69.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of the multivariate logistic regression test using the Backward Wald method showed two variables were the independent risk factors for GH/PE: age of ≥ 31.5 years and BLL of ≥ 35.15 g/dL (Table 3). To analyze the potential role of age, BMI, and BLL in increasing blood pressure, a correlation test was conducted between these variables and BP variables (systolic/diastolic blood pressures). The Rank-Spearman test showed a significant correlation between age and BMI (p < 0.001; r = 0.442), age and SBP (p < 0.001; r = 0.582), and age and DBP (p < 0.001; r = 0.491). Meanwhile, for BLL, although it was not significant, it was positively correlated with BMI (p = 0.067; r = 0.231), SBP...
Regarding exposure to lead, there is no safe limit for BLL in pregnant women and children (34). Even the slightest exposure to lead should be avoided. Exposure to lead in small but continuous doses (long term) harms the body as lead enters through the respiratory tract (inhalation) and the digestive tract (oral). It causes bioaccumulation of lead in the body which will be stored in the bones (35). In pregnant women, the need for calcium increases, especially to support fetal growth (36-37). When the calcium intake is inadequate, calcium cannot be maintained in the bones (bone decalcification). Followed by the release of lead in the bones, BLL will increase (38-39). The insufficient intake of calcium may be one of the causes of the high BLL in pregnant women in the research subjects.

GH/PE is a health problem in pregnant women whose pathophysiology is unclear (7,40). One of the causes is oxidative stress (41-42). Exposure to lead causes an increase in the production of Reactive Oxygen Species (ROS) which triggers the oxidative stress. Oxidative stress leads to endothelial function disorders and increased blood pressure. The increase in ROS causes the Nitric Oxide (NO), which is a vasodilator, to become inactive, thereby increasing intra-arterial pressure and lowering NO production (16). The disturbances in these three components (increased ROS, raise arterial pressure, and lower NO production) cause an increase in peripheral vascular resistance along with increased blood pressure (43).

The experimental studies in animals have shown that exposure to lead can increase plasma norepinephrine and downregulate adrenergic receptors, thereby stimulating the sympathetic nervous system and leading to an increased vasoconstrictive response (41). Lead also interferes with the function of the Na/K-ATPase pump which plays a role in maintaining intracellular calcium balance. It is able to affect the sodium gradient and the activity of the sodium/calcium exchanger, which increases intracellular calcium and the smooth muscle cells contractility (44). Several reviews have shown that pregnant women with GH/PE have low NO levels, increased ROS, and endothelial dysfunction (45-46). The positive relationship between GH/PE and BLL, proven by pathogenic studies, suggests that exposure to leads contributes to the pregnancy complications (11,12).

Besides, the current study also found other variables that could potentially became risk factors for GH/PE, namely maternal age and BMI. The cut-off value calculation for the age variable showed that age of > 31.5 years was a risk factor for GH/PE. Research proved...
that age over 35 years is a risk factor for PE (47). While the results of this study obtained a younger age limit (31.5 years old), this was probably due to differences in ethnicity and the risk of exposure to toxic substances in the environment.

Meanwhile, for the BMI variable, the gestational age and maternal height were relatively the same in both groups (GH/PE and normal pregnancy); the weight of the subjects in the case (GH/PE) group was greater, so was BMI. The difference in these variables between the groups was significant. This study revealed BMI of > 28.4 increased the risk of GH/PE. Several studies have shown that the excessive weight gain in pregnant women increases the risk of GH/PE (48-49). The excessive weight gain during pregnancy is likely associated with chronic inflammatory conditions, which surges plasma levels of C-reactive protein, inflammatory cytokines (50–52), and a systemic inflammatory response characterized by increased ROS that damages endothelium cells in blood vessels, resulting in clinical symptoms of GH/PE (53-54).

This study showed a positive correlation between BLL and BMI (Table 4). Similarly, a study in China found BLL is independently associated with BMI of women (55), while the other study concluded children whose mothers had elevated BLL (≥5.0µg/dL) are more likely to be overweight or obese (56). The increase in BMI because of exposure to lead is likely related to oxidative stress which disrupts endocrine function and body fat metabolism (57). Meanwhile, another theory proposes the alterations in the hypothalamic-pituitary-adrenal axis, stress-induced increase in glucocorticoid levels, oxidative stress, and the changes in lipid metabolism (49).

Lead is also hematotoxin which interferes with hemoglobin (Hb) synthesis, causing anemia via the inhibition of ferrochelatase and δ-aminolaevulinic acid dehydratase (ALAD) among many enzymes involved in heme biosynthesis (58). Several studies on the impact of exposure to lead on the blood system, both in adults and children, have been carried out. Research in India proved that BLL in pregnant women with anemia is greater than in non-anemic pregnant women (59). Anemia in pregnant women needs to be prevented because it causes impaired fetal growth, low birth weight or small for gestational age (60), and increased risk of maternal and perinatal mortality (61).

In addition to the occurrence of GH/PE, exposure to lead impacts the fetus, especially fetal growth and development (62-63). Research showed an increase of 1 µg/dl BLL was associated with changes in birth weight of −9.93 g, head circumference -0.03 cm, and crown-heel length of -0.05 cm (64). A systematic review found that exposure to lead is a risk factor for Attention Deficit Hyperactivity Disorder (ADHD) in children (65-66). Research in Korea revealed that children with BLL above 2.30 µg/dL were at a 2.5-fold greater risk of having ADHD (66). Another study concluded that maternal BLL in late pregnancy of < 5mg/dL affected the neurodevelopment of children up to 36 months of age (67). These results prove that even in low exposure, the toxic nature of lead is a serious problem for children’s growth and development.

To reduce maternal mortality, the Indonesian Ministry of Health needs to take into account environmental factors, e.g., lead as one of the toxic materials which can trigger the incidence of GH/PE in pregnant women. In high-risk areas for exposure to lead (e.g., industrial or agricultural areas), it is necessary to carry out BLL examination on pregnant women in the first trimester, especially in the age group above 30 years. Even earlier screening should be carried out to check BLL in women who have pregnancy planning.

Calcium supplementation programs for pregnant women also need to be encouraged. Several studies have shown that high doses of calcium supplementation (1200 mg/day) can reduce the BLL in pregnant women (68) and prevent the occurrence of GH/PE (69). The World Health Organization (WHO) recommends calcium supplementation (1,500-2,000 mg/day) in pregnant women from 20 weeks of gestation until the end of pregnancy. Especially, those who are at high risk of developing gestational hypertension should take the supplementation (70). The WHO also recommends calcium supplementation in pregnant women with BLL of ≥ 5µg/dL and inadequate calcium intake (71).

Adequate calcium intake during pregnancy will prevent the occurrence of inadequacy of calcium resources in the bones (bone decalcification) so that the release of lead from the bones into the circulatory system can also be prevented. Calcium intake also plays a role in the blood pressure regulation by modifying calcium in vascular smooth muscle cells and regulating blood vessel volumes through the renin-angiotensin-aldosterone system. The inadequate calcium intake will increase the activity of the parathyroid gland. The excessive parathyroid hormone also will increase intracellular calcium in vascular smooth muscle, which causes vasoconstriction and increases blood pressure (72).

Theoretically, the best effort to overcome the impact of exposure to toxic substances in the environment is reducing the use of these toxic materials, for example, reduced use of lead, oil, pesticide and others in the electronics industry, battery industry. Another effort is reducing the contamination-risk factors.
in water, air, soil, food/drink, and objects around us. Research showed that remediation efforts, e.g., reducing the lead content in the soil in the battery recycling site, can reduce the BLL in children living around this location (73). However, implementing some of these alternative solutions is not easy. Thus, early detection of BLL in pre-conception period is the best solution besides calcium supplementation. The American College of Obstetricians and Gynecologists (ACOG) stated that pregnant and lactating women with current or previous BLL of 5 µg/dL or higher should get the intervention related to nutritional intake, especially calcium and iron supplementation (74).

Based the discussion, there is high risk of exposure to lead in pregnant women on the North Coast of Java in Brebes District. It has an impact on increasing BLL and blood pressure (GH/PE). The limitation of this study is that there was no measurement of oxidative stress parameters, such as levels of ROS and NO, or endothelin which can more accurately describe the mechanism of GH/PE because of exposure to lead. The cross-sectional design used in this study also caused the temporality aspect to be uncertain, whether exposure to lead occurred before the occurrence of GH/PE was not identified. To analyze the impact of exposure to lead on the health of pregnant women, fetal growth, further cohort studies need to investigate delivery process, pregnancy outcomes, and even the quality of child development in the first trimester of pregnancy.

ACKNOWLEDGEMENTS

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CONCLUSION

The BLL in pregnant women with GH/PE is higher than normal pregnant women, and the BLL in the high category (≥ 35.15 µg/dL) is an independent risk factor for GH/PE. For better preventive efforts, BLL screening should be carried out in high-risk women or in the pre-conception period itself. Further, the calcium supplementation program can be done earlier, starting from the first trimester of pregnancy.

REFERENCES


