# Correlation Between Iron Status And Blood Pressure With Nitric Oxide (NO) Levels Of Pregnant Women In Semarang City, Indonesia

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# Correlation Between Iron Status And Blood Pressure With Nitric Oxide (NO) Levels Of Pregnant Women In Semarang City, Indonesia

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**Abstract---** *Cases of pregnant women's death are still high, and most are caused by bleeding and hypertension. However, studies that prove the relationship of iron status and blood pressure with Nitric Oxide levels for pregnant women are still limited. This study aims to analyze the relationship between hemoglobin level, serum transferrin receptor (sTfR), and blood pressure with nitric oxide level in pregnant women. This study used a cross-sectional design. Subjects were 79 pregnant women in their third trimester in Semarang City, Central Java Province, Indonesia. Hemoglobin level was measured with cyanomethamoglobin meanwhile serum transferrin receptor, and NO was measured with ELISA method. Data were analyzed with rank spearman. Study results reveal that the median of hemoglobin level was 11.30 g/dl, the median of sTfR was 15.06 nmol/l, and the median of NO was 63.69 nmol/l. There were no correlation between NO with hemoglobin level (p=0.776; r=0.033) and sTfR (p=0.568; r= -0.065). There was an inverse correlation between NO and systolic blood pressure (p= 0.014; r= -0.276), however there was no significant correlation between NO and diastolic blood pressure (p= 0.060; r= -0.212). There was a significant difference in the NO level between pregnant women with normal blood pressure and hypertension. Among several variables studied, NO levels are only correlated with systolic blood pressure.*

***Keywords:*** *Nitric oxide, serum transferrin receptor, hemoglobin, blood pressure, pregnant women.*

## I. INTRODUCTION

Maternal death cases are still a concern in many countries, especially in developing countries. The maternal mortality rate is still very high, according to the World Health Organization, there are 830 mothers worldwide who die from complications related to pregnancy and childbirth and 99% of all maternal deaths occur in developing countries [1]. It is estimated that in 2015, around 303,000 women died during and after pregnancy and childbirth. The leading cause is due to low resources, and most of it can be prevented [2]. Besides, the majority of cases of maternal mortality are due to bleeding cases and hypertension [3; 4; 5; 6; 7]. Previous studies have suggested that chronic hypertension increases the risk of maternal death 7.2-fold and increases the risk of prematurity, low birth weight, and low Apgar score [3]. Cases that cause the death of pregnant women such as iron-deficiency anemia often occur in rural areas [8].

Meanwhile, Indonesia, as part of a developing country, also noted that 38 mothers died every day due to cases of illness related to pregnancy and childbirth [1]. This condition provided an impetus for experts to conduct study of specific causes of maternal death. One of the special studies that need to be done is the relationship between iron status and blood pressure with levels of *Nitric Oxide* (NO) in pregnant women. Until now, studies that discuss the relationship between iron status and blood pressure with NO levels are still limited.

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Iron is an essential mineral for the synthesis of hemoglobin and myoglobin in a human body. During pregnancy, the requirement for absorbed iron increases from 0.8 mg/day in the first trimester to 7.5 mg/day in the third trimester. The average requirement for absorbed iron in the entire gestation period is 4.4 mg/day. Adequate iron status is essential for pregnant women and normal growth of the fetus. The absorbed iron is used for the expansion of pregnant woman's red cell mass as well as growth and development of organs in the fetus and placenta [9]. Anemia during pregnancy is still a global health problem that affects nearly half of all pregnant women worldwide. According to WHO, anemia during pregnancy is classified as severe if the hemoglobin level is below 7 g/dl, moderate if the hemoglobin level is between 7 to 9.9 g/dl and mild if the hemoglobin level is between 10 to 10.9 gr/dl [10]. Generally, the pathological cause of anemia during pregnancy is iron deficiency, which arises as a consequence of increased use of iron in the fetus [11]. Anemia during pregnancy increases perinatal risk and low birth weight infants [12].

Hemoglobin level is often used as pseudo-marker of iron deficiency. However, the hemoglobin level is not suitable to assess iron status, particularly during pregnancy [9]. Iron deficiency in tissue can be determined with biomarker *serum transferrin receptor* (sTfR), which is a single polypeptide chain protein contained in plasma. In contrast to plasma ferritin, plasma transferrin receptors are not affected by infection or inflammation; thereby, they can be used to differentiate iron deficiency anemia and anemia of chronic diseases [13]. A person is identified as having an iron deficiency if the sTfR level is equal to or more than 21.0 nmol/l [14].

*Nitric Oxide* (NO) is a biologic mediator synthesized from L-arginine. NO plays a role in physiological and pathological regulatory processes such as inflammation, vasodilation, and metabolism. It can increase the intracellular affinity of iron regulating proteins for elements that are responsive to iron in transferrin receptors and ferritin mRNA [15]. NO affects iron metabolism by distracting the iron-sulfur complex of iron regulating proteins. NO concentration in those with iron deficiency anemia increases gradually as iron deficiency develops and is three times higher than those with normal iron status. NO concentration has a positive correlation with sTfR, which is a sensitive indicator of erythropoiesis. It is related to low iron stores in those with severe iron deficiency anemia because the concentration of sTfR does describe not only erythropoiesis activity but also iron status. Hence positive correlation between NO concentration and sTfR is not caused by an increase of erythropoiesis activity but the severity of iron depletion [16]. In blood vessels, NO regulates blood vessel tone and blood flow by activating soluble guanylate cyclase (sGC) in vascular smooth muscle. Abnormalities in production and transport of NO to blood vessels cause endothelial dysfunction, such as hypertension [17].

Cases of pregnant women's death are still high, and most are caused by bleeding and hypertension. However, studies that prove the relationship of iron status and blood pressure with Nitric Oxide levels for pregnant women are still limited. Thus, this study aims to reveal the relationship between iron status and blood pressure with NO levels in pregnant women. Specifically, this study aims to analyze the correlation between hemoglobin level, sTfR, and NO in pregnant women.

## II. MATERIALS AND METHODS

This study used the observational method with a cross-sectional design where variables were measured at the same time. The subjects were 79 pregnant women in their third trimester from three community health centers at Semarang City, Central Java Province (see Table 1). Variables of this study were hemoglobin level, serum transferrin receptor (sTfR), and nitric oxide (NO) level in pregnant women. Ethical Clearance No.197/EC/FKM/2017 was obtained from the Commission of Ethics of Medical and Public Health Research, Faculty of Public Health, Diponegoro University.

Characteristics of Subjects

| Characteristics | N = 79 |
|---|---|
| Age (years) | 27.95 ± 5.08 |
| Gestational Age (months) | 8 (7 – 9) |
| Parity | 2 (1 – 3) |
| Weight (kg) | 50.0 (35.0 – 92.0) |
| Height (cm) | 152.85 ± 5.73 |
| Mid-Upper Arm Circumference (cm) | 25.0 (19.8 – 35.0) |
| Monthly Income (thousand rupiahs) | 2,000 (500 – 6,000) |
| Level of Education, n (%) | |
| Primary | 25 (31.6) |
| Secondary to Higher | 54 (68.4) |
| Blood Pressure (mmHg) | |
| Systolic | 114.87 ± 13.64 |
| Diastolic | 72.00 (56.00 – 98.00) |
| Hemoglobin (g/dl) | 11.30 (8.90 – 14.30) |
| sTfR (nmol/l) | 15.06 (8.59 – 34.94) |
| NO (nmol/l) | 63.69 (6.20 – 341.94) |

Subject characteristic data such as age, level of education, and monthly family income were obtained through interview method in Indonesian and Javanese language, which is a traditional language of the local area. Anthropometric measurements were conducted to obtain data on body weight, height, and mid-upper arm circumference (MUAC) of pregnant women. Bodyweight was measured using a digital scale with an accuracy of 0.1 kg, height was measured using stature meter with an accuracy of 0.1 cm, and MUAC was measured using MUAC tape. Maternal blood pressure was measured using a digital sphygmomanometer with the Duplo method (twice measurements). Hypertension was defined as a systolic pressure of >120 mmHg and a diastolic pressure of >80 mmHg [18]. Data of nutrition intake was obtained through 24-hours recall method for two in consecutive days. Food intake was recorded on 24-hours recall form in household portion (plates, cups, tablespoons, and teaspoons), then converted into grams to analyze the nutritional content using Nutrisurvey software. Data of education level was classified as primary education and secondary to higher education. Subjects with primary education have studied at least nine years consisting of primary school or equivalent for six years and junior high school or equivalent for three years [19]. Meanwhile, secondary to higher education was defined as three years of education in high school or equivalent and four years in college.

About 5 ml of venous blood was taken to examine the hemoglobin level, sTfR, and NO. Hemoglobin level was measured with cyanomethamoglobin meanwhile, both sTfR and NO were measured with the ELISA method. Reagent kit used were Biovendor-Laboratorni Medivina, Karsek, Czech Republic (Cat: RD194011100) for sTfr measurement and R&D Systems, Inc., Minneapolis, USA (Cat: KGE001) for NO level measurement. sTfR and NO level were measured using Microplate Reader Biorad 680 model (Bio-rad Laboratories Inc, CA, USA) with software Microplate Manager version 5.2.1 (Bio-rad Laboratories Inc, CA, USA). The measurement was held in Prodia Laboratory.

Statistical analysis was conducted using SPPS software ver. 23. Based on the normality test with Kolmogorov-Smirnov, data on hemoglobin, sTfR, and NO level of pregnant women were analyzed using rank spearman test with 95% significance level to determine the correlation between variables. Mann-Whitney test was used to compare between NO level of pregnant women with their normal blood pressure and hypertension.

## III. RESULTS

In this study, the mean age of subjects was 28 years, while the median of gestational age was eight months. Median of monthly family income was 2 million rupiahs approximately. There were 68.4% subjects with secondary to higher education level and 31.6% with primary education. Mean of systolic blood pressure was 114.87 mmHg, and a median of diastolic blood pressure was 72 mmHg. Median of sTfR and NO were 15.06 nmol/l and 63.69 nmol/l respectively.

Rank spearman test showed that there were no significant correlation between NO level with hemoglobin (p = 0.776) and sTfR (p = 0.568). NO level had a negative correlation with systolic blood pressure (p = 0.014; r = -0.276) but no significant correlation with diastolic blood pressure (p = 0.060) (see Tabel 2).

Correlation between NO Levels with Hemoglobin, sTfR, and Blood Pressure in Pregnant Women

| Correlation between Variable | R | p |
|---|---|---|
| Correlation between NO and hemoglobin | 0.033 | 0.776 |
| Correlation between NO and sTfR | -0.065 | 0.568 |
| Correlation between NO and blood pressure | | |
| NO and systolic blood pressure | -0.276 | 0.014 |
| NO and diastolic blood pressure | -0.212 | 0.060 |

Maternal blood pressure was classified into hypertension and normal to know the difference of NO level between both groups. Mann-Whitney test found a significant difference in NO levels between pregnant women who had hypertension and those with normal blood pressure (p = 0.036). Most pregnant women in this study had normal blood pressure (59.5%), while the rest of them had hypertension (40.5%) (see Table 3).

The Difference in NO Levels between Hypertensive and Normotensive Pregnant Women

| Category of Blood Pressure | n | % | p |
|---|---|---|---|
| Hypertension | | | |
| Elevated | 13 | 16.5 | |
| Stage 1 | 13 | 16.5 | 0.036 |
| Stage 2 | 6 | 7.5 | |
| Normal | 47 | 59.49 | |
| Total | 79 | 100.00 | |

## IV. DISCUSSION

This study found that there was no correlation between NO level with hemoglobin level and sTfR. It is caused by an increase in the number of hormones during pregnancy. Maternal nitric oxide metabolite concentrations increased during pregnancy and declined before the labor at term. Nitric oxide can be synthesized from L-arginine via two constitutive calcium-sensitive isoforms of nitric oxide synthase (NOS), i.e., endothelial NOS (eNOS) and neuronal NOS (nNOS), or an inducible isoform (iNOS) which is calcium-independent. In uterine tissue of experimental animal (e.g., rats and rabbits), nitric oxide production was reported to be increased and would be decreased at term.

On the contrary, in human myometrium, NOS isoforms expression and the possibility of pregnancy-associated changes in their expression are still debatable [20]. Estrogen hormones are vasoactive hormones that can initiate rapid vasodilation in various vascular sites and perfusion of tissues throughout the body. The effects of rapid vasodilation of estrogen in the uterus are affected by arterial endothelial production from a vasodilator NO [21].

Another study in women aged 14-19 years showed that there was a correlation between NO levels and hemoglobin and sTfR [16]. NO levels of subjects with iron depletion were significantly higher than healthy subjects as controls. NO level

increased gradually as iron deficiency anemia developed and is three times higher than control when it is clear that they suffer from iron deficiency anemia. NO level is 7.5 times higher in those with severe iron deficiency (Hb <80 g / l) than those with high hemoglobin levels (Hb ≥ 140 g / l) [16].

This study found that there was a correlation between NO and systolic blood pressure of pregnant women. It is related to the increase in the volume of blood stored in the cardiovascular system by systemic vasodilation during pregnancy. Vasodilation involves an increase in NO production, thereby reducing peripheral vascular resistance in healthy pregnant women. On the other hand, low NO production causes hypertensive disorders of pregnancy, such as preeclampsia and gestational hypertension [22]. In multiparous women with chronic hypertension, maternal serum NO levels are lower than in nulliparous. This is allegedly due cardiovascular burden of some previous pregnancies that affect the cardiovascular system and result in an increase of endothelial damage, thereby reducing NO production [23].

Blood pressure is generally lower in women than men and often decreases during pregnancy when estrogen and progesterone hormone increases. NO synthesis, which is the primary regulator of vasodilation is higher in women than men. An increase in NO production by vascular endothelial cells induces relaxation of surrounding vascular smooth muscle cells, resulting in vasodilation and decrease blood pressure [24].

This study also found a significant difference in NO level between hypertensive and normotensive pregnant women. A study in 28 weeks pregnant women showed a different result where NO excretion between hypertensive and normotensive pregnant women was not different significantly. However, there was a direct correlation between urinary NO, excretion, and a change in systolic blood pressure on hypertensive pregnant women between first-trimester antenatal booking visit and the third trimester [25]. A decrease in glomerular capillary function results in the release of essential substances such as renin, angiotensinogen, angiotensin I, angiotensin II, angiotensin-converting enzyme (ACE), aldosterone, bradykinin, and NO which ultimately leads to increased blood pressure. NO reduction also causes Na retention or increased sodium reabsorption (renal pressure natriuresis). This situation will increase total peripheral resistance and cardiac output and cause hypertension [26; 27].

In conclusion, this study showed that were no significant correlation between NO with hemoglobin and sTfR, which was allegedly related to an increase in estrogen and progesterone hormones during pregnancy. We also found that NO levels and systolic blood pressure had a negative correlation. Pregnant women with hypertension and normotensive had significantly different NO levels.

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