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The Effect of Oral and Intravenous Iron Supplementation on Serum Hepcidin and Ferritin Levels in *Rattus Norvegicus* Pregnant Strain Wistar with Anemia

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ABSTRACT

Background: Anemia is an indirect cause of high maternal mortality rates worldwide characterized by low levels of hemoglobin, hepcidin, and serum ferritin. Hepcidin is a biomarker of iron metabolism in the body while ferritin acts as a store of iron. Hepcidin and ferritin levels during pregnancy will decrease. To restore the levels, supplementation is required either orally or intravenously. This study aimed to determine the effect of oral and intravenous iron supplementation on hepcidin and ferritin levels.
Methods: This was an experimental laboratory study using *Posttest Only Control Group Design*. The sample was 24 *Rattus Norvegicus* divided into 4 groups: the negative control group without treatment, the positive control group given NaNo, the, P1 given oral iron supplementation, and the P2 given intravenous iron. The maintenance of the test animals in this study was carried out at the Pharmacy laboratory of Universitas Andalas Padang in June-August 2021. The experimental animals were *Rattus Norvegicus* Wistar females weighing 200-250 grams, aged between 3-4 months conditioned to be pregnant. The serum examination of hepcidin and ferritin levels used the ELISA method and the normality test used *Shapiro Wilk*. Then, the significance test used One-Way ANNOVA followed by multiple comparisons of Bonferroni types.
Results: The results showed that there were significant differences ($p<0.05$) in hepcidin levels in the positive control group, treatment group 1, treatment 2 that were 219.52 ng/ml, 220.27 ng/ml, and 221.49 ng/ml. Likewise, the ferritin levels in the positive control group, treatment 1, and treatment 2 were 5.91 ng/ml, 6.81 ng/ml and 7.72 ng/ml.
Conclusion: Based on the findings, it can be concluded that oral and intravenous iron supplementation had an effect on increasing serum hepcidin and ferritin levels in *Rattus norvegicus* pregnant strain Wistar with anemia.

Keywords: *Oral iron supplementation, intravenous supplementation, hepcidin and ferritin*

INTRODUCTION

The data from *World Health Organization* (WHO) in 2018 Maternal Mortality Rate (MMR) in the world is 210/100,000 Live Births (KH) which around 295,000 mothers die due to complications during pregnancy and childbirth. Of this figure, almost 94% of maternal deaths occur in developing countries and 40% of maternal deaths are directly related to anemia during pregnancy. (1)

Maternal mortality itself is caused by direct and indirect causes. Indirect causes are Chronic Energy Deficiency in

pregnancy and anemia. (2) Anemia can be caused by various things, including iron deficiency. Based on *Riskesdas* in 2018, there were about 61.9% of mothers consuming iron during pregnancy but only 38.1% who received iron tablets of more than 90 grains. (3)

According to the results of *Riskesdas*, the coverage of giving Fe tablets to pregnant women in Indonesia in 2018 was 73.2%. It has not reached the 2018 Strategic Plan target of 95%. In West Sumatra Province, it has a coverage rate of 79.93% and has not yet reached the Strategic Plan target. West Sumatra occupies the 12th lowest position in the coverage of giving Fe tablets to pregnant women. (3)

Iron (Fe) supplementation is a blood-added tablet useful for tackling anemia. Iron is one of the most important components in the body. (4) Giving iron tablets is expected to prevent bleeding during childbirth, reduce maternal mortality, and increase nutritional intake for the fetus to prevent stunting. (3)

Nowadays, in overcoming the problem of anemia in pregnant women, it is still focused on giving iron tablets or known to the public by giving Blood Add Tablets. Pregnant women receive 90 Fe tablets during pregnancy. (5) Meanwhile, consideration in giving iron supplementation intravenously has not been carried out. (6)

The presence of hepcidin levels which is one of the biomarkers to see the presence of iron deficiency plays a role in maintaining iron levels in the body. Apart from being a regulator of iron levels in the blood, it is also known to act as a mediator in anemia. In this case, hepcidin can perform its function through its binding to ferroportin (FPNI), resulting in internalization and degradation of FPN1 which will inhibit iron transport from three sources, namely hepatocytes which will affect the level of iron reserves (ferritin) in hepatocytes, then in enterocytes which will affect absorption of iron in the intestine and macrophages that affect iron recycling. A situation where hepcidin levels increase, then hepcidin will inhibit the release of iron from these three sources so that it will cause a lack of iron carried into the plasma and result in anemia (7).

METHODS

Design and sampling

This is an experimental laboratory study using *Posttest Only Control Group Design*. The sample was 24 *Rattus Norvegicus* divided into 4 groups: the negative control group without treatment, the positive control group given $NaNo_2$, the P1 given oral iron supplementation, and the P2 given intravenous iron. The maintenance of the test animals in this study was carried out at the Pharmacy laboratory of Universitas Andalas Padang in June-August 2021. The experimental animals were *Rattus Norvegicus* Wistar females weighing 200-250 grams, aged between 3-4 months conditioned to be pregnant. The examination of serum hepcidin and ferritin levels used the ELISA method and the normality test used *Shapiro Wilk.* The significance test used One-Way ANNOVA followed by multiple comparisons of Bonferroni types.

Treatment dose

A total of 28 rats divided into 4 treatment groups were conditioned first to be anemic, each cage containing 1 male and 7 female mated. The negative control group was not given any treatment, only received aquabidest by feeding. Meanwhile, the positive control group was given $NaNo_2$ 3.75 ml/200 g body weight, same as the oral treatment group and the intravenous treatment group. However, the negative control group was only given regular food and drink and did not receive iron supplementation in oral form, given with a sonde with an oral dose 1.2-1.5 cc for *Rattus Norvegicus* body weight with a body weight 200-250 gr for 14 days and intravenously by intravenous injection with an intravenous dose 0.9-1.125 cc for *Rattus Norvegicus*

body weight with a body weight 200-250 g given for 14 days.

Furthermore, it measured the levels of *hepcidin* and *ferritin* post supplementation with the procedure of taking *Rattus Norvegicus* blood under anesthesia, then 3 ml of blood was drawn through the medial canthus of the orbital sinus using a non-EDTA hematocrit capillary pipette. To obtain plasma, the blood was then centrifuged at 3500 rpm for 5 minutes. The plasma was packaged by using *dry ice* and sent to the biomedical laboratory of Universitas Andalas for the serum examination of hepcidin and ferritin levels using the method *Enzyme Linked Immunosorbent Assay* (ELISA) kit.

Data analyze

The data were analyzed to see the normality by using *the Shapiro Wilk test* with (p> 0.05) then continued with the One-Way ANNOVA test to see the relationship between variables. Furthermore, the analysis was continued with the *Multiple Comparison* test (post hoc test) of the *Bonferroni* type to see the differences between groups.

RESULTS

Hepcidin

Table1: hepcidin mean levels on each group based on treatment

Groups	Mean (ng/ml) ± Std. Deviation	P value
K -	221,16 ± 0,79	0,002
K +	219,52 ± 0,56	
P 1	220,27 ± 0,80	
P 2	221,49 ± 1,06	

The mean level of hepcidin in the K-group was 221.16 ng/ml. In K+, the mean hepcidin level was lower than the other groups, which was 219.52 ng/ml. The P1 group had a mean 220.27 ng/ml and the P2 group had a mean 221.49 ng/ml. Based on the One-Way ANNOVA, there was a significant difference between the control group and the treatment group on hepcidin levels with a value of p=0.002 (p<0.05).

Ferritin

Table2: ferritin mean levels on each group based on treatment

Group	Mean (ng/ml) ± Std. Deviation	P value
K (-)	7,27 ± 0,48	0,001
K (+)	5,91 ± 0,48	
P 1	6,81 ± 0,43	
P 2	7,72 ± 0,33	

The average ferritin level in the K-group was 7.27 ng/ml. In K+, the mean ferritin level was lower than the other groups, which was 5.91 ng/ml. The P1 group had a mean 6.81 ng/ml and the P2 group had a mean 7.72 ng/ml. Based on the One-Way ANOVA, there was a significant difference between the control group and the treatment group on ferritin levels with p value = 0.001 (p <0.05).

DISCUSSION

The excessive administration of $NaNo_2$ will cause several negative effects on the body, especially due to tissue hypoxia. The low concentration of hepcidin due to hypoxia is also thought to be a stabilizing mechanism by the liver through *hypoxia-inducible factor* (HIF)-1, reducing the effect of the BMP/SMAD signaling pathway. Increased HIF activity is associated with increased hemojuvelin cleavage activity *by* matriptase, so this will decrease hepcidin expression. Another role of HIF can be seen in mice with intestinal HIF2 deficiency, which have low expression of *ferroportin* and DMT1, and cannot absorb iron well, even with low hepcidin levels. Therefore, HIF is thought to play a role in the regulation of the hepcidin gene either due to hypoxia or low iron levels. (7)

In functional iron deficiency, inflammation increases hepatic hepcidin expression via IL6-JAK2-STAT3 signaling leading to reduce FPN abundance and function in cells, depriving plasma iron. In response to iron deficiency anemia, the kidneys produce erythropoietin, stimulating erythropoiesis. Erythropoietin sensitivity of erythroblasts can be modulated by TfR2. In absolute iron deficiency, erythroblasts and erythrocytes donate iron via FPN-mediated iron export. Increasing the erythropoiesis (e.g., during recovery from anemia) causes

the secretion of erythroferron, suppressing hepatic hepcidin expression via inhibition of BMP-SMAD signaling (red pathway). LSEC = hepatic sinusoidal endothelial cells. P = phosphorylated. TSAT=transferrin saturation. (8)

Oral administration of iron supplements affects the gut microbiome, increasing inflammation so that it is not maximally absorbed by duodenal cells. (9) Meanwhile, the intravenous administration directly into the blood vessels will accelerate the erythropoiesis process so that it is directly related to the increase in hemoglobin levels. (10) This increase in Hb affects the increase in hepcidin regulating iron regulation in blood plasma whose mechanism works together with ferroportin. In addition, IV supplementation can reduce the risk of gastrointestinal toxicity, avoid oxidative damage to the intestinal mucosa, with a rapid increase in Hb, and will be positively correlated with increasing serum hepcidin levels. (11) (25)

Zehra (2017) found that intravenous iron supplementation causes an increase in hepcidin levels. Iron deficiency anemia is associated with low serum hepcidin levels. This is due to a complex series of reactions resulting in decreasing the hepcidin production and increasing the iron absorption. (12)

Hepcidin content is a 25-amino-acid protein with eight cysteine residues and 4 disulfide bonds, presented in many species. The expression of hepcidin is encoded by the HAMP gene, which can produce 84-amino-acid preprohormone which will become the mature hormone hepcidin-25. (7) (24)

In its work, hepcidin has the opposite mechanism with ferroportin serving as an iron exporter in the cell membranes of macrophages, hepatocytes, and enterocytes. Hepcidin can stimulate the internalization and degradation of ferroportin, leading to increase the intracellular iron storage, decrease the iron absorption, and decrease the circulating iron levels. Because of this role, hepcidin is also considered as a substance that can indirectly play a role in the body's defense. This is because hepcidin can reduce the amount of iron circulating in the plasma while iron is an important material for bacterial growth. Thus, hepcidin can be bacteriostatic. (7) (24)

In non-anemic conditions, when iron stores are adequate or high, hepcidin will be released and bind to intestinal ferroportin causing internalization and destruction of ferroportin. The decreased ferroportin activity causes absorbed iron to remain in enterocytes. On the other hand, when iron stores are low, the production and secretion of hepcidin is suppressed, thereby increasing the release of iron from enterocytes into the blood plasma (10)

However, overcoming iron deficiency anemia in pregnancy is not easy. Patient adherence to daily oral iron treatment regimens was low, most likely due to the high frequency of gastrointestinal side effects, as well as the low systematic absorption rate of oral iron. In addition, the optimal regimen of oral iron treatment is unclear. Therefore, an alternative method of intravenous administration is needed. (13)

$NaNo_2$ can bind to hemoglobin and cause the formation of reactive oxygen species (ROS). ROS causes oxidative stress on the erythrocyte membrane and erythrocytes to be unable to maintain their elasticity, and then hemolysis occurs (14). Nitrite ions absorbed in the blood enter the erythrocytes, and oxidize^{+} to Fe3, resulting in the formation of methemoglobin. The formed methemoglobin cannot transport oxygen so that the cells will lack oxygen supply and hypoxia occurs, and it it will cause a decrease in ferritin which will lead to anemia. (15)

Oral administration of iron supplementation taken between meals and its absorption can be inhibited by foods containing calcium and drugs reducing stomach acidity. With consistent supplementation, reticulocytes can be started in 4-5 days, and Hb improves in week 2. Oral iron therapy is required for at

least 3-6 months to meet iron stores and normalize ferritin levels. (16) (23)

Meanwhile, the intravenous supplementation does not have an absorption phase so that the drug enters the bloodstream directly. It has 100% bioavailability with a fast and efficient onset. (17) As a result, giving iron intravenously more quickly restores iron stores in the form of ferritin and causes an increase in hemoglobin levels compared to oral administration. (22)

Ferritin is a protein consisting of 22 apoferritin molecules while the core consists of a phosphate/iron complex of 4000–5000 iron molecules per nucleus. Ferritin is soluble in water and a small amount is soluble in plasma. The greater the amount of ferritin, the more dissolved in the plasma. Ferritin levels for men are 40–300 g/L and 20–150 g/L for women. (18)

Kumari et al (2020) compared the effectiveness of intravenous iron sucrose versus oral iron in the treatment of iron deficiency anemia during pregnancy. The study revealed that the intravenous iron sucrose is a safe and effective alternative to oral iron in treating anemia, iron deficiency in pregnancy. (19)(21)

In addition, Dalal (2018) found that oral iron therapy is influenced by many factors such as limited absorption, low tolerability, non-adherence, and side effects. Then, the increase in hemoglobin levels was more common in mothers who were given iron intravenously than given orally. (20).

CONCLUSION

It can be concluded that oral and intravenous iron supplementation had an effect on increasing serum hepcidin and ferritin levels on *Rattus Norvegicus* pregnant strain Wistar with anemia.

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