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Original Article

Effects of time course ferrous sulphate supplementation on iron regulation in pregnant rats

Zahrah Zakiyah, M.Midwifery [a,*], Yunda D. Jayanti, M.Midwifery [b], Nurdiana Nurdiana, PhD [c] and Pande M. Dwijayasa, PhD [d]

[a] *Faculty of Health Sciences, University of Respati Yogyakarta, Yogyakarta Special Region, Indonesia*
[b] *Dharma Husada Midwifery Academy, Kediri, East Java, Indonesia*
[c] *Pharmacology Laboratory, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*
[d] *Department of Obstetric and Ginecology, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

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الملخص

أهداف البحث: تهدف هذه دراسة لتقييم تأثير مكملات كبريتات الحديد على تنظيم وتوازن الحديد في الفئران الحوامل.

طرق البحث: تم تقسيم ٢٤ من الفئران الحوامل لأربع مجموعات؛ بما فيها مجموعة التحكم (دون علاج)، ومجموعة الحوامل التي أخذت كبريتات الحديد ابتداء من الثلث الأول من الحمل (اليوم الأول للحمل)، الثلث الثاني (اليوم الثامن من الحمل)، والثلث الثالث (اليوم ١٥ من الحمل). أعطيت كبريتات الحديد عن طريق الفم بواسطة أنبوب تغذية فموي حتى الولادة. وقيس الحديد في الدم وقدرة ارتباط الحديد الكلية بطريقة القياس اللونية. كما قيست مستويات الهيبسيدين باستخدام طريقة المقايسة المناعية.

النتائج: زاد الحديد في الدم، وتشبع الترانسفيرين، ومستويات الهيبسيدين بشكل كبير في المجموعة التي أعطيت كبريتات الحديد في الثلث الثالث من الحمل بالمقارنة بالثلث الثاني أو الأول من الحمل، أو في الثلث الثالث من الحمل عن الثلث الثاني. بينما نقصت بشكل كبير مستويات قدرة ارتباط الحديد الكلية في المجموعة التي أخذت كبريتات الحديد في الثلث الأول من الحمل بالمقارنة بالثلث الثاني أو الثالث. ونقصت مستويات قدرة ارتباط الحديد الكلية أيضا بشكل كبير في المجموعة التي أخذت كبريتات الحديد في الثلث الثاني من الحمل بالمقارنة بالثلث الثالث.

الاستنتاجات: إعطاء كبريتات الحديد مبكرا في الحمل يؤدي إلى مستويات أعلى للحديد في الدم، وتشبع الترانسفيرين، والهيبسيدين.

الكلمات المفتاحية: الحمل؛ الحديد في الدم؛ ارتباط الحديد؛ الهيبسيدين؛ الترانسفيرين

Abstract

Objectives: Our study aimed to evaluate the effects of ferrous sulphate supplementation on iron regulation and homeostasis in pregnant rats.

Methods: Twenty-four pregnant rats were divided into four groups; including the control (untreated) pregnant group and the pregnant groups that received ferrous sulphate starting at the 1st trimester (1st day of pregnancy), 2nd trimester (8th day of pregnancy), and 3rd trimester (15th day of pregnancy). Ferrous sulphate was administered orally with an oral gavage until birth. Serum iron and total iron binding capacity were measured by a colorimetric method. Hepcidin levels were measured using an immunoassay method.

Results: The serum iron, transferrin saturation, and hepcidin levels were significantly increased in the group given iron sulphate in the 3rd trimester compared with the 2nd or 1st trimesters and in the 3rd trimester compared with the 2nd trimester ($p < 0.05$). The total iron binding capacity levels were significantly decreased in the group that received iron sulphate in the 1st trimester compared with the 2nd or 3rd trimesters ($p < 0.05$). The total iron binding capacity levels were also significantly decreased in the group that received iron sulphate in the 2nd trimester compared with the 3rd trimester ($p < 0.05$).

Conclusions: Early administration of ferrous sulphate in pregnancy leads to higher levels of serum iron, transferrin saturation, and hepcidin.

* Corresponding address: University of Respati Yogyakarta, Jl. Raya Tajem km 1.5 Maguwoharjo, Depok, Sleman, Yogyakarta, Yogyakarta Special Region, Indonesia.
E-mail: zahrah.zakiyah85@gmail.com (Z. Zakiyah)

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Keywords: Hepcidin; Iron binding; Pregnancy; Serum iron; Transferrin

Introduction

During pregnancy, the systemic iron requirement increases 10-fold to support placental and foetal growth.[1] Iron requirements increase during pregnancy due to the expansion of the maternal erythrocyte mass and the high demand for iron in the growing foetus. These requirements are initially met through mobilization of maternal iron stores (principally from the liver), but as iron stores become depleted, intestinal iron absorption increases to maintain an adequate iron supply for both the mother and her offspring.[2,3] The foetus obtains its iron via the placenta, which sequesters transferrin-bound iron from maternal circulation. The rate of maternal-foetal transfer increases with the increasing size of the foetus and placenta and is maximal just prior to parturition.[4,5] Iron absorption is also maximal at this time.[6] Foetal and neonatal iron deficiency results in decreased growth, immunological dysfunction, anaemia, and irreversible cognitive defects.[7]

Iron supplementation is highly recommended to prevent iron deficiency anaemia during pregnancy.[1] The bioavailability and iron absorption from the daily diet are influenced by the type and quantity of iron present in food, as well as by the presence of inhibitors and promoters of iron absorption in the diet and the individual's iron status.[8] Several biomarkers have been used to assess the iron status in individuals. These include haemoglobin, serum ferritin, zinc protoporphyrin, total iron-binding capacity, and transferrin saturation.[9] For most living organisms, iron is essential, but potentially toxic, making the maintenance of systemic iron homeostasis critical. This homeostasis is orchestrated by the hormone hepcidin, which regulates the levels of the cell membrane iron exporter ferroportin. Hepcidin binds to ferroportin, inducing its degradation and leads to decreased iron availability.[10] Therefore, this study aimed to investigate the effect of a time course of ferrous sulphate supplementation on iron regulation in pregnant rats.

Materials and Methods

Animals

Twenty-four pregnant female rats (*Rattus norvegicus*), age 8 weeks, weight 100–200 g were divided into four groups, including the control (untreated) pregnant group and the pregnant groups that received ferrous sulphate starting at the first trimester (1st day of pregnancy), second trimester (8th day of pregnancy), and third trimester (15th day of pregnancy). This study was conducted at the Pharmacology Laboratory, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia.

Ferrous sulphate treatment

Ferrous sulphate powder was created using a mortar and was dissolved in water (60 ml volume containing 300 mg). Ferrous sulphate treatment was performed on the 1st day of pregnancy, 8th day of pregnancy, and 15th day of pregnancy. Ferrous sulphate was administered orally with an oral gavage until birth.

Serum collection

At the end of the experiment, the rats were anesthetized with ketamine intramuscular injection, and then the serum was obtained. All samples were stored at −80 °C until used for analysis.

Serum iron and total iron binding capacity analysis

A commercial colorimetric serum iron and total iron binding capacity detection kit (Quantichrom Iron Assay Kit, Catalogue No: DIFE-250, BioAssay System) was used to measure serum iron and total iron binding capacity levels in the serum sample.

Hepcidin analysis

A commercial hepcidin detection kit (Cussabio, catalogue No. CSB-ELO10124RA) was used to measure hepcidin levels in the serum sample.

Statistical analysis

Data are presented as the mean ± SD, and the differences between groups was analysed using one-way analysis of variance (ANOVA) with SPSS 16.0 statistical package for Windows. Only the probability values of $P < 0.05$ were considered to be statistically significant and later subjected to Post hoc test.

Results

Table 1 presents the serum iron levels in the various experimental groups. The levels of serum iron were significantly greater in all three groups treated with ferrous sulphate compared with the control group ($p < 0.05$). The serum iron levels were significantly decreased in the group given the iron sulphate in the third trimester compared with the second or first trimesters ($p < 0.05$). The serum iron levels were also significantly lower in the group treated with the iron sulphate in the second trimester compared with the first trimester ($p < 0.05$). Thus, higher levels of the serum iron will result when iron sulphate is given earlier in pregnancy.

The total iron binding capacity levels in the control group and the groups administered iron sulphate during pregnancy is shown in Table 1. The total iron binding capacity levels was significantly lower in all treatment groups compared with the control group ($p < 0.05$). The total iron binding capacity levels were significantly decreased in the group given iron sulphate in first trimester compared with the

second or third trimesters ($p < 0.05$). The total iron binding capacity levels were also significantly decreased in the group given the iron sulphate in the second trimester compared with the third trimester ($p < 0.05$). Thus, the earlier iron sulphate is given in pregnancy, the lower the levels of the total iron binding capacity will be.

Table 1 also presents the transferrin saturation levels in the various experimental groups. The levels of transferrin saturation were significantly greater in all three groups treated with ferrous sulphate compared with the control group ($p < 0.05$). The transferrin saturation levels were significantly increased in the group given iron sulphate in first trimester compared with the second or third trimesters ($p < 0.05$). The transferrin saturation levels were also significantly higher in the group treated with the iron sulphate in the second trimester than in the third trimester ($p < 0.05$). Thus, the earlier the iron sulphate is given in pregnancy, the higher the levels of transferrin saturation will be.

Table 2 presents the serum hepcidin levels in the various experimental groups. The levels of serum hepcidin were significantly greater in all three groups treated with ferrous sulphate compared with the control group ($p < 0.05$). The serum hepcidin levels were significantly increased in the group given the iron sulphate in the first trimester compared with the second or third trimesters and in the second trimester compared with the third trimester ($p < 0.05$). Thus, the earlier in pregnancy the iron sulphate is given, the higher the levels of serum hepcidin will be.

Discussion

Iron homeostasis results from a complex set of events that start with the absorption of iron by the intestinal cells, its transport into the cell, and its further release into the blood stream, where it is transported by carrier proteins (i.e., transferrin) and stored in different body stores, which are mainly bone marrow, liver, and spleen (known as ferritin complexes).[11,12] In states of iron overload or supplementation, the iron level will be high, and the TIBC will be low or normal, causing the transferrin saturation to increase. In this study, the earlier ferrous sulphate is given during pregnancy, the higher the levels of serum iron and transferrin saturation will be. This shows that the administration of ferrous sulphate starting at the first trimester of pregnancy can increase iron absorption through the intestine, which causes increased levels of serum iron and protein carrier. Total iron binding capacity is the ability of the blood to bind iron with transferrin. In other words, total iron binding capacity reflects the amount of iron that can be transported in the blood. Total iron binding capacity was calculated from the sum of measured unsaturated iron-binding capacity (UIBC) and measured serum iron (i.e., TIBC = UIBC + serum iron).[13] In this study, administration of iron sulphate in the first trimester has a value of total iron binding capacity that is significantly lower than the second and third trimesters. In other words, the earlier ferrous sulphate is given, the lower the total iron binding capacity will be. Our findings are consistent with previous studies that revealed that during pregnancy, sTfR responds to iron supplementation when there is iron-deficiency anaemia.[14]

Maternal hepcidin concentrations were significantly correlated with indicators of maternal iron status.[15–17] During the first trimester of pregnancy, serum and urinary hepcidin were positively correlated with ferritin and negatively correlated with serum transferrin receptor (sTfR) index, a sensitive indicator of iron deficiency.[16] Similarly, throughout the gestational period, serum hepcidin correlated positively with ferritin and transferrin saturation and negatively with sTfR and haemoglobin concentration.[17] This finding suggests that hepcidin regulation by iron and erythropoiesis is preserved in pregnancy.[18] In our study, the levels of serum hepcidin were significantly greater in all three groups treated with ferrous sulphate compared with the control group

Table 1: Levels of serum iron, total iron binding capacity, and transferrin saturation in each experimental group.

	Control	Ferrous sulphate supplementation		
		1st trimester	2nd trimester	3rd trimester
SI (mg/L)	30.70 ± 7.42	178.49 ± 49.20[a]	109.60 ± 4.55[ab]	75.01 ± 4.19[abc]
TIBC (mg/L)	1674.50 ± 98.90	498.30 ± 105.17[a]	690.60 ± 127.87[ab]	1313.00 ± 149.45[abc]
TS (%)	0.018 ± 0.0034	0.3549 ± 0.049[a]	0.1625 ± 0.260[ab]	0.0575 ± 0.0039[abc]

Note: Data are presented as the mean ± SD; [a]$p<0.05$; in comparison with the control group; [b]$p < 0.05$; in comparison with ferrous sulphate supplementation in the 1st trimester group; [c]$p < 0.05$; in comparison with ferrous sulphate supplementation in the 2nd trimester group; SI: serum iron; TIBC: total iron binding capacity; TS: transferrin saturation; m/L: milligramme/litre.

Table 2: Levels of serum hepcidin in each experimental group.

	Control	Ferrous sulphate supplementation		
		1st trimester	2nd trimester	3rd trimester
Hepcidin (ng/mL)	510.43 ± 62.86	1209.37 ± 112.60[a]	936.78 ± 185.90[ab]	744.62 ± 163.50[abc]

Note: Data are presented as the mean ± SD; [a]$p<0.05$; in comparison with the control group; [b]$p < 0.05$; in comparison with ferrous sulphate supplementation in the 1st trimester group; [c]$p < 0.05$; in comparison with ferrous sulphate supplementation in the 2nd trimester group; ng/mL: nanogramme/millilitre.

($p < 0.05$). The serum hepcidin levels were significantly increased in the group given the iron sulphate in the first trimester compared to the second or third trimesters and in the second trimester compared with the third trimester ($p < 0.05$). Thus, the earlier in pregnancy the iron sulphate is given, the higher levels of the serum hepcidin levels will be. Our finding indicates that the increase in iron status from ferrous sulphate supplementation will reduce iron demand. Our study is consistent with previous studies that demonstrate that the increase in iron demand during pregnancy is met by an adaptive decrease in maternal hepcidin levels, which leads to enhanced iron absorption. This is a highly conserved process observed in humans and animal models.[6,15,19]

In conclusion, the earlier the beginning of ferrous sulphate is given in pregnancy, the higher the levels of serum iron, transferrin saturation, and hepcidin will be.

Authors' contributions

ZZ, YDJ, NN, and PMD conceived and designed the study, conducted research, provided research materials, and collected and organized data. ZZ, YDJ, and PMD analysed and interpreted data. ZZ, YDJ, and NN wrote the initial and final drafts of article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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