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Serum iron level after ingestion of repeated dose of iron shortly after and 2 hours after meal

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ABSTRACT

Erna Kristin, Mohammad Hakimi, Sri Kadarsih Soejono, Lukman Hakim - ***Serum iron level after ingestion of repeated dose of iron shortly after and 2 hours after meal***

Background: The prevalence of iron deficiency anemia in pregnant women is estimated to be higher than non-pregnant women. Iron supplementation program has been proven clinically to improve the hemoglobin (Hb) level of pregnant women. The absorption of iron is affected by food, therefore iron has to be given 2 hours after meal. However, in practice, iron was given shortly after meal. The physiological change in pregnant women affects drug absorption, distribution, and elimination phases.
Objective: To understand serum iron level after ingestion of repeated dose of iron shortly after and 2 hours after meal for 12 weeks in trimester 2 pregnant women with iron deficiency anemia.
Method: The research design was a phase II clinical trial. Subjects were 24 trimester 2 pregnant women with iron deficiency anemia, classified into two groups, who were treated as follows: The first group was consisted of 12 women with iron deficiency anemia, treated with twice-a-day ferrous sulphate tablet @ 300 mg orally, given shortly after meal for 12 weeks; the second group was consisted of 12 women with iron deficiency anemia, treated with twice-a-day ferrous sulphate tablet @ 300 mg orally, given 2 hours after meal for 12 weeks. Blood samples were taken in week 2, 4, 6, 8, 10, and 12 after treatment. Serum (ferric) iron level was measured with Vitros Fe Slides method.
Result: Minimum, maximum, and average steady-state iron levels (Css min, Css max, Css average) of treatment 1 were 104.1 ± 14.03 ug/dL, 96.44 ± 13.22 ug/dL, and 112.38 ± 14.03 ug/dL (mean ± SEM), respectively; while minimum, maximum, and average steady-state iron levels (Css min, Css max, Css average) of treatment 2 were 125.77 ± 9.31 ug/dL, 118.03 ± 9.21 ug/dL, and 125.77 ± 9.31 ug/dL (mean ± SEM), respectively. No statistical significant difference was found within treatment in minimum steady-state level between week 2, 4, 6, 8, 10, 12 after treatment. There was also no significant difference in minimum steady-state level between treatment groups in week 2, 4, 6, 8, 10, and 12.
Conclusion: There were no differences in serum iron level after ingestion of repeated dose of iron shortly after and 2 hours after meal for 12 weeks in trimester 2 pregnant women with iron deficiency anemia.

Keywords: iron supplementation - serum iron - iron deficiency anemia - steady - state iron level

ABSTRAK

Erna Kristin, Mohammad Hakimi, Sri Kadarsih Soejono, Lukman Hakim - ***Kadar besi serum setelah pemberian tablet besi dosis berulang selama 12 minggu yang diberikan sesaat dan 2 jam setelah makan pada wanita dengan anemia defisiensi besi***

Latar Belakang: Prevalensi anemia defisiensi besi pada ibu hamil diperkirakan lebih tinggi dibandingkan dengan ibu yang tidak hamil. Program suplementasi besi terbukti secara klinis meningkatkan kadar Hb ibu hamil. Absorpsi sediaan besi sudah terbukti dipengaruhi oleh makanan, sehingga pemberian obat dilakukan minimum 2 jam setelah

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makan. Namun dalam prakteknya obat sering diminum sesaat setelah makan. Perubahan fisiologis wanita hamil memberikan pengaruh pada fase absorpsi, distribusi dan eliminasi obat.
Tujuan: Mengetahui kadar besi serum setelah pemberian tablet besi dosis berulang selama 12 minggu yang diberikan sesaat setelah makan dan 2 jam setelah makan pada wanita hamil trimester 2 dengan anemia defisiensi besi.
Metode: Penelitian dilakukan dengan rancangan uji klinik fase II. Jumlah subyek 24 wanita hamil trimester 2 dengan anemia defisiensi besi yang dibagi dalam dua kelompok dan mendapat perlakuan sebagai berikut: Kelompok pertama terdiri dari 12 orang, yang diberi perlakuan 2 kali sehari tablet fero sulfat @ 300 mg per oral, diberikan sesaat setelah makan, selama 12 minggu. Kelompok kedua, juga 12 orang, diberi perlakuan 2 kali sehari tablet fero sulfat @ 300 mg per oral, diberikan 2 jam setelah makan, selama 12 minggu. Sampel darah diambil pada minggu ke 2, 4, 6, 8, 10 dan 12 setelah perlakuan. Kadar besi dalam serum (sebagai ferri) ditetapkan dengan cara Vitros Fe Slides.
Hasil : Rata-rata kadar besi tunak minimum, maksimum dan rata-rata (Css min, Css max, Css average) pada perlakuan 1 berturut-turut sebesar 108,78 ± 13,79 ug/dl, 121,44 ± 15,79 ug/dl, 116,11 ± 13,13 ug/dl (mean ± SEM). Sedangkan rata-rata kadar besi tunak minimum, maksimum dan rata-rata (Css min, Css max, Css average) pada perlakuan 2 berturut-turut sebesar 115,15 ± 46,27 ug/dl, 141,36 ± 61,36 ug/dl dan 124,92 ± 53,43 ug/dl ((Mean + SEM). Perbedaan nilai rata-rata kadar besi tunak, maksimum dan rata-rata pada masing-masing perlakuan dan antara dua perlakuan tidak bermakna secara statistik ($p > 0,05$).
Simpulan: Tidak terdapat perbedaan parameter kadar besi serum setelah pemberian tablet besi dosis berulang selama 12 minggu yang diberikan sesaat setelah makan dan 2 jam setelah makan pada wanita dengan anemia defisiensi besi.

INTRODUCTION

The prevalence of iron deficiency anemia in pregnant women is estimated to be higher than non-pregnant women. Iron deficiency anemia may be occurred at the end of pregnancy, although the woman did not have anemia at the beginning of pregnancy. It is estimated that half of pregnant women worldwide have iron deficiency anemia. In Indonesia, the prevalence of anemia in pregnant women is reported to be around 43.6%.[1]

Iron supplementation program for pregnant women had been successful in developed countries. Sweden has implemented iron supplementation and food fortification program for years and the prevalence of iron deficiency anemia was relatively low.[2]

CDER suggested that food might change the bioavailability of drugs in various ways, such as prolonging the stomach emptying, stimulating bile production, changing the pH, or directly affecting the physicochemical properties of drugs. Food that affects the drug bioavailability also has potential to affect its clinical aeffect. The absorption of iron has been proven to be affected by food. Since drug absorption is affected by food, drug has to be given for a minimum of 2 hours after meal.[3] However, in practice, drug is taken shortly after meal.

Several studies showed that drug intake in pregnant women might cause different response, compared with non-pregnant women. It was caused by the physiological change in pregnancy. This difference was caused by the change in pharmacokinetic parameters in pregnat women, such as slower absorption rate, lower plasma protein level, higher volume of distribution of hidrophylic and liphophilic drugs resulted from the increase in plasma volume and body fat, and higher renal clearance.[4] The change in pharmacokinetic parameters for several drugs results in inappropriate dose given to pregnant women. The difference in response to drugs in pregnant women may be the cause of inadequate result in iron supplementation program.

National Program of ferro sulphate tablet supplementation for pregnant women with iron deficiency anemia suggested that 3 tablet a day is given for 90 days in pregnancy and until 42 days after birth.[5] Iron tablet is given in the form of ferro sulphate or ferro fumarate tablet in a certain dose usually given to pregnant women with Hb level < 11 gr/dL, but in the direction of use, there is no explicit time for taking the drug, whether they were taken in first, second, or third trimester of pregnancy.[6] It has been known that in pregnant women, there is a diffference in pharmacokinetic profile in second trimester, therefore, a study is needed to understand the iron bioavailability in repeated dose iron tablet supplementation for second trimester pregnant women given shortly after and 2 hours after meal.

METHOD

This research was conducted in Kecamatan Sleman, Pakem, Turi, Seyegan, Tempel, Kabupaten Sleman, in August 2003 until July 2004. The design was phase II clinical trial.

Subjects of the study were 24 second trimester pregnant women with iron deficiency anemia.[2] Sample size was calculated based on CDER guideline which suggested that minimum sample size for drug bioavailability study were 12.[3]

The inclusion criteria for subjects were: 1) second trimester pregnant women with Hb level < 11 gr/dL and ferritin level < 12 ng/mL, identified through active surveillance, who agreed to participate in the study by signing informed consent; 2) age of the subjects were between 19-39 years old; 3) body height > 145 cm; 4) bodyweight > 40 kg; 5) upper arm circumference > 21.5 cm; 6) has given birth once; 7) diet screening showed diet score > 75%; and 8) lived in the area of study for a minimum of 3 months.

Individuals who had given birth more than three times, had a history of allergy, had smoking or drinking alcohol habit, had malaria, worm disease, renal disorder, had a history of heart and liver diseases, and/or had taken other iron preparations were excluded from this study.

Protocol of the study had been approved and ethical clearance had been given by Ethical Committee on Biomedical Research on Human, Faculty of Medicine, Gadjah Mada University.

Subjects were classified into two groups and treated as follows: The first group was consisted of 12 second trimester pregnant women with iron deficiency anemia, treated with twice-a-day ferrous sulphate tablet @ 300 mg orally, given shortly after meal for 12 weeks; the second group was consisted of 12 second trimester pregnant women with iron deficiency anemia, treated with twice-a-day ferrous sulphate tablet @ 300 mg orally, given 2 hours after meal for 12 weeks. Tablets were given each week to the subjects.

In this study, outcomes measured were serum ferric iron level taken every 2 weeks (week 2, 4, 6, 8, 10, and 12 after treatment), which was expected to represent minimum, maximum, and average steady-state iron levels (Css min, Css max, Css average). Blood samples taken shortly before taking drugs represented minimum steady-state level and samples taken 1 hour after taking drugs represented maximum steady-state level. To obtain an illustration of several parameters of food taken by subjects, food consumption survey was conducted 3 times, in week 2, 6, and 10.

Serum (ferric) iron level was measured with Vitros Fe Slides method. Serum iron level and pharmacokinetic parameter values from serum data obtained from first and second groups were compared using t-test with significance level of 95%.

RESULT

Average age of the subjects were 28.8±3.82 years old, the youngest was 20 years old and the oldest was 35 years old. Average body height was 151.65 cm and average bodyweight was 55.22 kg. Average Hb level before intervention was 9.87 ± 0.15 g/dL, while average ferritin level was 5.42±0.62 µg/dL. Average upper arm circumference was 26.52±3.11 cm and average BMI was 24.07±3.83.

To obtain an illustration of several parameters of food taken by subjects, food consumption survey was conducted 3 times, in week 2, 6, and 10.

TABLE 1. Average food contents on food consumption surveys in week 2, 6 and 10

Food contents	Treatment		p
	1	2	
Ferritin	15.48	19.28	0.732
Vitamin C (mg)	153.88	89.09	0.021
Zinc	6.81	7.76	0.274
Protein (g)	52.49	54.33	0.674
Calcium (mg)	577.96	415.38	0.114
Carbohydrate (g)	269.80	246.91	0.299
Heme iron (mg)	79.09	90.02	0.568
Fermented food	137.95	240.70	0.563
Phytic acid (mg)	44.19	59.6	0.848
Tannin (mg)	0.90	3.52	0.103

There was no significant difference between groups in the food contents, consisted of ferritin, zinc, protein, carbohydrate, heme iron, fermented food, phytate, and tannin (TABLE 1).

One of the markers of drug availability in blood after repeated dose administration is steady-state level. Iron steady-state level after repeated dose administration in second trimester pregnant women in group 1 and 2 is shown in TABLE 2. Generally,

plasma iron steady-state levels between both groups were not different in all observation period, that is, week 2, 4, 6, 8, 10, and 12 after treatment. Lowest minimum steady-state iron level was seen at week 0 (before treatment), that is, 105.64 µg/mL in group 1 compared with 85.08 µg/mL in group 2. Iron minimum steady-state levels before treatment were not different between both groups.

TABLE 2. Average (mean±SEM) values of steady-state iron level (minimum, maximum, average) in week 0, 2, 4, 6, 8, 10, and 12 after ferrous sulphate tablet @ 300 mg twice a day administration for 12 weeks in subjects with iron deficiency anemia (n=24)

Week	Iron level (ug/dL) (mean±SEM)		p
	Treatment group 1 (n=12)	Treatment group 2 (n=12)	
0 (before treatment)			
Minimum steady-state level	105.64 ± 16.39	85.08 ± 11.54	NS
2 (after treatment)			
Minimum steady-state level	109.92 ± 15.67	116.82 ± 8.74	NS
Maximum steady-state level	116.75 ± 15.50	137.18 ± 7.82	NS
Average steady-state level	113.34 ± 15.59	127 ± 8.28	NS
4 (after treatment)			
Minimum steady-state level	88.50 ± 10.21	111.09 ± 11.68	NS
Maximum steady-state level	106.67 ± 11.84	128.67 ± 13.39	NS
Average steady-state level	97.59 ± 11.03	119.88 ± 12.54	NS
6 (after treatment)			
Minimum steady-state level	102.00 ± 11.39	110.92 ± 7.02	NS
Maximum steady-state level	113.83 ± 13.54	127.25 ± 6.79	NS
Average steady-state level	107.92 ± 12.47	119.09 ± 6.91	NS
8 (after treatment)			
Minimum steady-state level	95.82 ± 14.48	109.17 ± 8.65	NS
Maximum steady-state level	113.00± 19.47	124.83 ± 7.40	NS
Average steady-state level	104.41 ± 16.98	117 ± 8.03	NS
10 (after treatment)			
Minimum steady-state level	86.64 ± 9.82	129.50 ± 13.63	NS
Maximum steady-state level	107.00 ± 14.57	143.83 ± 14.89	NS
Average steady-state level	96.82 ± 12.20	136.67 ± 14.26	NS
12 (after treatment)			
Minimum steady-state level	95.73 ± 17.75	130.67 ± 5.52	NS
Maximum steady-state level	117.00 ± 14.02	139.22 ± 6.20	NS
Average steady-state level	106.37 ± 15.89	134.95 ± 5.86	NS

NS=not significant

Compared with group 1, maximum and minimum steady-state iron levels in group 2 were higher from week 2 until week 12, however, the difference was not significant. The difference in maximum and minimum iron levels in group 2 were relatively constant for 12 weeks.

The average of minimum, maximum, and average steady-state iron level in week 0, 2, 4, 6, 8, 10, and 12 is shown in TABLE 3.

TABLE 3. Average values of minimum, maximum, and average steady-state iron levels in week 0, 2, 4, 6, 8, 10, and 12 after ferrous sulphate @ 300 mg twice a day administration for 12 weeks in subjects with iron deficiency anemia

Parameter	Treatment group 1 (n=12)	Treatment group 2 (n=12)	p
Minimum steady-state level (mean±SEM)	104,41± 14,03	125,77 ± 9,31	NS
Maximum steady-state level (mean±SEM)	96,44 ± 13,22	118,03 ± 9,21	NS
Average steady-state level (mean±SEM)	112,38 ± 14,03	125,77 ± 9,31	NS

TABLE 3 showed that minimum, maximum, and average steady-state iron levels in group 2 were higher than those in group 1, but the difference was statistically insignificant.

DISCUSSION

Measurement of iron level after repeated dose administration to pregnant women is needed, because bioavailability test specified by Center for Drug Evaluation and Research used single dose and conducted on non-pregnant women.[3] While applying pharmacokinetic parameters obtained from bioavailability test on pregnant women, we need to realize that there was a difference in pharmacokinetic parameter values in pregnant women compared with non-pregnant women. The change in cardiovascular function is occurred in pregnancy, the increase in blood volume and the decrease in serum protein level may significantly change the pharmacokinetic of several antibiotics in pregnant women, therefore dose determination based on data obtained from non-pregnant women can not be applied directly on pregnant women.[4]

Moreover, individual variation in serum levels after standard dose administration is far wider in pregnant women compared with non-pregnant women and men. This difference is not associated with age, body weight, or gestational age. Although accurate information on dose for pregnant women is very limited, a change in dose administered may be needed if the serum level have to be maintained at adequate level to give satisfying therapeutical effect.

Physiological change in pregnancy, started from first trimester, and most obvious in third trimester, may change the drug absorption, distribution, and clearance. Bowering has conducted a study on pregnant women in East Harlem and result showed serum average iron level of 84 μg/dL.[7] Cross-sectional study on 318 pregnant women in West Java by Suharno *et. al.* showed average iron level of 56.42 μg/dL.[8]

Variation of iron levels in pregnant women with anemia obtained from several studies showed that iron level may be used directly to find out the iron status of pregnant women. Several studies showed that iron level in pregnant women was relatively low compared to that in non-pregnant women. To obtain a more objective illustration, comparation have to be conducted at the same period and population, like the study by Johnson *et. al.* who showed that iron level in reproductive and non-pregnant women was 98.5 μg/dL, while that in pregnant women was 88.4 μg/dL.[9] Similar result was observed if the result was compared with iron level of pregnant women in the study by Suharno *et. al.*, which was much lower than the result in this study.[8]

In this study, serum average iron level in second trimester pregnant women before treatment (110.57 μg/dL) was higher than the level reported Dallman *et. al.* and Suharno *et. al.*[8,10] When comparing group 1 and 2, average steady-state level in group 2 (125.77 μg/dL) was higher than group 1 (112.38 μg/dL). The higher iron level in group 2 showed that iron absorption might be better in group 2. Iron absorption estimation in pregnant women, particularly in second trimester, could not be analogized to the iron absorption in non-pregnant women. The difference in the time of iron tablet administration in pregnant women had an effect on the high blood iron level, although the difference was not significant. One of the method that may be used to explain it is by detecting the iron absorption rate using radioisotope-labelled iron.[11-12]

During pregnancy, there is physiological change that may also changes the drug absorption and pharmacokinetic parameters. Stomach emptying may be slower and intestinal motility is decreased. The increase in stomach pH is occurred because of the decrease in H^- secretion and the increase in mucus secretion. When there is no physiological change, iron absorption is occurred best in intestinum that has neutral pH. In pregnancy, iron given with food may stayed in stomach with higher pH. An increase in pH may also occurred, caused by the decrease in H level and the increase in mucus secretion, so that stomach pH become neutral and iron absorption is occurred. The decrease in intestinal motility also causes the increase in iron absorption. Meanwhile, when iron is administered two hours after meal, the increase in pH in stomach is not high enough to provide appropriate condition, so that iron absorption is not occurred in the stomach.[4]

The change in the effect of iron supplementation on blood iron level of second trimester pregnant women was caused by the physiological change during pregnancy. The effect of iron supplementation caused the increase in iron level, but in second trimester, the increase was not shown. It was caused by the change in volume of distribution, so that Cmax was apparently lower. The lower Cmax was resulted from the expansion of intravascular (plasma volume) and extravascular (mammary gland, uterus, peripheral oedema) water content, that may increase the Vd.[4] Besides, Lynch showed that iron absorption in second trimester was relatively lower. Pregnant women had complex physiological change that might increase or decrease the iron absorption.[13]

Pharmacokinetic approach in this study could not directly used to explain the change in iron absorption process and could not answer directly why iron tablet given 2 hours after meal resulted in lower iron level compared with the same drug given shortly after meal. Important information that may be gathered from this study is that pharmacokinetic study conducted on non-pregnant women could not be directly used to estimate the effect of iron tablet in pregnant women.

CONCLUSION

There was no differences in iron serum levels after ferrous sulphate tablet @ 300 mg twice a day administered shortly after meal and 2 hours after meal for 12 weeks in trimester 2 pregnant women with iron deficiency anemia.

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