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Serum iron level shortly after iron supplementation shortly after and 2 hours after meal in women with iron deficiency anemia

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ABSTRACT

Erna Kristin, Muhammad Hakimi, Sri Kadarsih Soejono, Lukman Hakim - ***Serum iron level shortly after iron supplementation shortly after and 2 hours after meal in women with iron deficiency anemia***

Background: Incidence of anemia in women in developing countries is still high, that is, around 43%. This incidence rate is far more higher that that in industrial countries, which is ranged between 10-12%. The prevalence of iron deficiency anemia is still high, particularly in developing countries. The cause of the high prevalence of iron deficiency anemia is not known, since it involves various factors. Two of the probable etiologic factors is variability in dosage administration, and the effect of co-administered food. Studies on the pharmacokinetic of iron after single dose iron tablet administration in women with anemia and pharmacokinetic of iron coadministered with food in healthy women have been done, but study on repeated dose has never been conducted.
Objective: To understand serum iron level after ingestion of repeated dose of iron shortly after and 2 hours after meal for 12 weeks in women with iron deficiency anemia.
Method: The research design was a fase II clinical trial. Subjects were 24 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 108.78 ± 13.79 ug/dL, 121.44 ± 15.79 ug/dL, and 115.11 ± 13.13 ug/dL (mean ± SEM), respectively; while minimum, maximum, and average steady-state iron levels (Css min, Css max, Css average) of treatment 2 were 115.15 ± 46.27 ug/dL, 141.36 ± 61.36 ug/dL, and 124.92 ± 53.43 ug/dL (mean ± SEM), respectively. No statistical significant difference were 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 group 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 women with iron deficiency anemia.

Key Words: iron supplementation-serum iron-iron deficiency anemia-steady-state iron level

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ABSTRAK

Kristin, E, Hakimi, M, Soejono, S.K., Hakim, L - ***Kadar besi serum setelah pemberian pemberian tablet besi dosis berulang selama 12 minggu yang diberikan sesaat dan 2 jam setelah makan pada wanita dengan anemia defisiensi besi***

Latar Belakang: Insidensi anemia pada wanita di negara sedang berkembang masih cukup tinggi, yaitu sekitar 43%. Angka ini jauh lebih tinggi daripada di negara-negara industri yang hanya berkisar antara 10-12%. Prevalensi anemia defisiensi besi sampai saat ini masih tinggi, terutama di negara sedang berkembang. Penyebab masih tingginya prevalensi anemia defisiensi besi ini belum diketahui secara pasti karena melibatkan banyak faktor. Salah satu faktor yang mungkin merupakan penyebab adalah tidak seragamnya aturan pakai atau adanya pengaruh makanan yang diberikan bersamaan. Penelitian mengenai farmakokinetika besi setelah pemberian tablet besi dosis dosis tunggal pada wanita anemia yang diberikan bersama makanan pada wanita sehat sudah pernah dilakukan, tetapi penelitian dengan pemberian dosis berulang belum pernah dilakukan.
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 dengan anemia defisiensi besi
Metode: Penelitian dilakukan dengan rancangan uji klinik fase II. Jumlah subyek 24 wanita dengan anemia defisiensi besi yang dibagi dalam dua kelompok dan mendapat perlakuan sebagai berikut: Kelompok pertama terdiri dari 12 wanita dengan anemia defisiensi besi, diberi perlakuan 2 kali sehari tablet fero sulfat @ 300 mg per oral, diberikan sesaat setelah makan, selama 12 minggu. Kelompok kedua, 12 wanita dengan anemia defisiensi besi, 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 penelitian: 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, 115,11 ± 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

Incidence of anemia in women in the developing countries is still high, that is, around 43%. This incidence rate is still higher than that in the industrial countries, which is ranging between 10-12%. Most anemia patients suffer from iron deficiency anemia, which is generally detected by the low serum ferritin level. Various randomized controlled clinical trial had consistently proven that iron supplementation might increase the iron status. The prevalence rate of women with low Hb and hematocrit levels decreased in group who received iron supplementation. In addition, serum ferritin level, serum iron level, and almost all variables associated with iron status, including bone marrow iron increase compared with control.[1,2]

Iron deficiency anemia is usually reversible and may be managed by iron supplementation.[3,4,5] However, the prevalence of iron deficiency anemia is still high, particularly in the developing countries.[6] The cause of the high prevalence of iron deficiency anemia is not known, since it involves various factors.[7] Two of the probable factors is the variability in dosage regimens and the effect of co-administered food.[3,4,7]

A drug will show pharmacological effect if it reaches its site of action in a certain concentration level. The concentration level in its site of action is directly affected by its concentration level in systemic circulation. The measurement of drug concentration in its site of action is not easy, therefore, the drug concentration in blood may be used as the most practical parameter to determine the dosage regimen, frequency, etc, by assuming that the concentration in blood reflects the concentration in the site of action.[8,9] Parameters used to find out the iron serum level of repeated dose drug administration are: steady-state drug concentration (Css min, Css max, Css average).[8,9,10]

In repeated dose drug administration, plasma drug level will achieve steady-state level in 3 times half-life, and then it fluctuates around average steady-state level. Average steady-state level is determined by maintenance dose and drug clearance. Plasma level fluctuation is determined by dosage interval and $T_{1/2}$. The shorter the dosage interval compared with $T_{1/2}$, the smaller the fluctuation, which is quantified by maximum/minimum ratio.[9,10]

Studies on pharmacokinetic profile of iron after single dose iron tablet administration given shortly after meal compared with 2 hours after meal, coadministered with food in women with iron deficiency anemia, had been conducted.[11] The study showed that in healthy women, there were no differences in iron serum level pharmacokinetic parameter values after ferrous sulphate 300 mg tablet administration given shortly after and 2 hours after meal. Study on pharmacokinetic profile of repeated dose iron after 12 weeks in women with iron deficiency anemia had never been conducted. Therefore, a study that aims to find out serum iron level after the administration of repeated dose iron tablet shortly after meal and 2 hours after meal for 12 weeks in women with iron deficiency anemia is needed.

METHODS

This study was conducted in 5 subdistricts of Sleman District, i.e Sleman, Pakem, Turi, Seyegan, Tempel. This phase II clinical trial was conducted between August 2003 and July 2004.

Subjects were 24 women with iron deficiency anemia.[12] The sample size determination was based on CDER guideline which determined that in drug bioavailability studies, the total subjects of each treatment group was 12.[13]

Subjects were included in this study if they met the following inclusion criteria: 1) women with Hb level < 12 g/dL and ferritin level < 15 ng/mL, identified with active surveillance, and those who agreed to participate in this study by signing informed consent; 2) age between 19-39 years old; 3) ≥ 145 cm height; 4) ≥ 40 kg weight; 5) upper arm circumference of the subject was ≥ 21.5 cm; 6) had given birth at least once; 7) diet score on screening was ≥ 75%; 8) had resided in the Kecamatan to be studied for a minimum of 3 months, and 8) used hormonal contraceptive method.

Women who had given birth more than three times, who had history of allergy, smoking or drinking alcohol, had malaria, worm infection, and renal disease at the time of examination, had a history of heart and hepatic diseases were excluded from the study. Protocol of the study has received ethical clearance from the Ethic Committee, Faculty of Medicine, Gadjah Mada University.

Subjects were classified into 2 groups, and received the following treatments: The first group 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 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. Tablets were distributed to subjects weekly.

Outcomes were serum iron level, measured every 2 weeks (week 2, 4, 6, 8, 10, and 12 after treatment), expected to reflect minimum, maximum, and average steady-state iron levels (Css min, Css mx, Css average), and might be used as the basis to quantify pharmacokinetic parameters, that is, half-life ($T_{1/2}$), clearance (Cl), and volume of distribution (Vd). Blood samples were taken shortly after drug administration to reflect minimum steady-state level and 1 hour after drug administration to reflect maximum steady-state level. To obtain the illustration of several parameters of food taken by subjects, food consumption surveys were conducted 3 times, at 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 treatment 1 and 2 were compared using t test, with significance level of 95%.

RESULT

The characteristics of the subjects were shown in TABLE 1. The average age of the subjects were 33.7 years old, with the youngest and the oldest were 22 years and 39 years old respectively. The proportion of subjects who had one survived child (29.2%) was smaller than that of subjects who had more than one survived children (70.7%). Average upper arm circumference values were 26.35±3.02 cm, and average BMI was 23.89±3.31.

TABLE 1. Characteristics of research subjects (n=24).

Characteristics	
Age (years)	
Mean±SD	33.7±5.5
Minimum	22
Maximum	39
Parity	
1	7(22.58)
>1	17(60.71)
Upper arm circumference (cm)	
Mean±SD	27.48±3.35
Body Mass Index	23.48±4.12

TABLE 2 shows that there were no significant differences in food contents between treatments, that was, in intake of ferritin, zinc, protein, carbohydrate, heme iron, fermented food, phytate, and tannin.

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 administered twice a day for 12 weeks in the treatment group 1 and 2 are shown in TABLE 3. The lowest minimum steady-state iron level was observed in week 0 (before treatment), that was, 90.50 ug/dL in the treatment group 1 compared with 103.00 ug/dL in the treatment group 2. There was no statistical significant difference in minimum steady-state iron level between both groups before treatment.

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

Food contents	Treatment		p
	1	2	
Ferritin	18.50	18.54	0.993
Vitamin C (mg)	52.52	108.99	0.048
Zinc	6.05	6.13	0.931
Protein (g)	54.97	54.62	0.972
Calcium (mg)	415.76	487.26	0.352
Carbohydrate (g)	252.23	240.89	0.527
Heme iron (mg)	74.01	68.23	0.889
Fermented food	333.38	292.99	0.773
Phytic acid (mg)	43.20	42.10	0.954
Tannin (mg)	1.89	0.93	0.275

TABLE 3. 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	90.50 ± 14.34	103.00 ± 18.18	NS
2 (after treatment)			
Minimum steady-state level	113.08 ± 21.77	155.25 ± 29.25	NS
Maximum steady-state level	116.89 ± 14.85	184.63 ± 47.33	NS
Average steady-state level	114.99 ± 18.31	149.94 ± 36.17	NS
4 (after treatment)			
Minimum steady-state level	113.83 ± 15.77	98.56 ± 17.17	NS
Maximum steady-state level	113.09 ± 20.10	116.08 ± 15.97	NS
Average steady-state level	113.46 ± 17.94	107.32 ± 16.57	NS
6 (after treatment)			
Minimum steady-state level	99.33 ± 7.97	84.09 ± 10.17	NS
Maximum steady-state level	125.25 ± 16.59	122.58 ± 18.56	NS
Average steady-state level	112.29 ± 12.28	103.34 ± 14.37	NS
8 (after treatment)			
Minimum steady-state level	102.17 ± 7.65	118.33 ± 65.96	NS
Maximum steady-state level	115.33 ± 9.39	139.42 ± 77.83	NS
Average steady-state level	108.75 ± 8.52	128.88 ± 71.90	NS
10 (after treatment)			
Minimum steady-state level	129.33 ± 19.56	111.91 ± 87.19	NS
Maximum steady-state level	139.55 ± 23.84	143.00 ± 121.08	NS
Average steady-state level	134.44 ± 21.7	127.46 ± 104.14	NS
12 (after treatment)			
Minimum steady-state level	94.92 ± 9.99	122.75 ± 67.48	NS
Maximum steady-state level	118.50 ± 9.95	142.42 ± 87.37	NS
Average steady-state level	106.71 ± 9.97	132.59 ± 77.43	NS

NS=not significant

After treatment 1 and 2, each group showed variation in minimum steady-state iron level. In the treatment group 1, the highest minimum steady-state iron level was achieved in week 10 after treatment (129.33 ug/dL). The treatment group 2 achieved their highest minimum steady-state iron level much earlier, i.e. in week 2 after treatment (155.25 ug/dL). Even though the minimum steady-state iron levels were smaller in the treatment group 1 compared with treatment group 2, no statistical significant difference were 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 both treatment groups in week 2, 4, 6, 8, 10, and 12.

Variation on maximum steady-state iron level were also found both in the treatment group 1 and 2. In the treatment group 1, the highest maximum steady-state iron level was achieved in week 10 after treatment (139.55 ug/dL). While in the treatment group 2, the highest maximum steady-state iron level was achieved much earlier, i.e. in week 2 after treatment (184.63 ug/dL). Variation of maximum steady-state iron level was smaller in the treatment group 1 than group 2, but no statistically significant difference were found within treatment group in week 2, 4, 6, 8, 10, and 12. Similarly, no statistically difference was also found between the treatment group 1 and 2 in week 2, 4, 6, 8, 10, and 12. It was also occurred in the variation of average steady-state iron level in the treatment 1 and 2.

Average values of minimum, maximum, and average steady-state iron levels in week 0, 2, 4, 6, 8, 10, and 12, and pharmacokinetic parameters quantified from average values was shown in TABLE 4.

TABLE 4. 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)	108.78 ± 13.79	115.15 ± 46.27	NS
Maximum steady-state level (mean±SEM)	121.44 ± 15.79	141.36 ± 61.36	NS
Average steady-state level (mean±SEM)	115.11 ± 13.13	124.92 ± 53.43	NS

It was shown in Table 4 that minimum, maximum, and average steady-state iron levels (Css min, Css max, Css average) in treatment 1 were lower than treatment 2, but the differences were not statistically significant.

DISCUSSION

In this study, iron level was used as the parameter of the bioavailability of iron after iron supplementation with the different administration time, that was, shortly after meal and 2 hours after meal. Bioavailability studies are usually used to measure drug levels in the body. Pharmacokinetic parameters are used as the basis of dosage regimen, dosage interval, and the assumption of therapeutic level. For many years the preferred technique of assessing iron status, in the absence of obvious anemia, was by measurement of transport iron in the serum. In the range of modest deficits in hemoglobin, the serum iron responds much more sharply than does hemoglobin. Across this range of major depletion to moderate anemia, these measures appear quite sensitive to the body supply of mobilizable iron. At higher levels of body iron, serum iron is still responsive but with much less sensitivity.[14]

Studies by Samman *et al* and Hunt used radioisotope labelled iron.[15,16] Samman *et al* conducted a study with the aim to prove that Chinese green tea and rosemary extraxt might significantly inhibit the nonheme iron absorption.[15] Hunt conducted a study with the aim to find out the iron absorption from diet with low and high bioavailabilities.[16]

The serum level of drugs given for a regular extended time with a certain interval will be either increased or decreased. The increase or decrease in drug concentration in serum will be affected by the association between half-life and dosage interval. If the drug administered has been completely

eliminated before the next dosage is given, repeated dose with constant interval will give similar serum levels. Indicator used to find it out is steady-state level. Steady-state level might be achieved because of the elimination process, which depended on dosage, the higher the increase in drug level, the faster the drug is eliminated per time unit. After several dosage administration, the drug level will reached a certain level where the total drug intake and elimination per time unit is equivalent, and this is called steady-state level. The level will increased or decreased in certain range of time proportional with the dosage administered and dosage interval.

In this study, discussion will be emphasized on the minimum steady-state level, because the average time to achieve peak level (Tmax) is 4 hours, while in this study, it was technically difficult to take blood sample after drug administration. Samples taking 1 hour after drug administration is more feasible. The consequence is the probability of very wide variations in maximum steady-state level, because the absorption process is not complete.

In this study, the average iron level was 96.75 mg/dL, higher than that reported by Toe & Than in healthy women (88 ug/dL).[17] It was also higher that that reported by Fricker *et al* in non-anemic healthy women (89 ug/dL).[18]

The average increase in iron level after supplementation was 12.08 mg/dL. The average increase in iron level in the treatment 1 (4.42 ug/dL) was lower than that in the treatment 2 (19.75 ug/dL). It showed that iron tablet administration 2 hours after meal would give higher serum level than iron tablet administered shortly after meal. This was similar with the result of a study by Hallberg & Hulthén who suggested that food decreased iron absorption.[19]

The average steady-state iron level (Css average) in this repeated dose study was a lot lower than Cmax in single dose, that was 274.25 ug/dL in treatment 1 and 232.92 ug/dL in treatment 2.[11] It also showed high absorption at the initial iron tablet administration. In repeated dose administration, regular iron intake for an extended time showed consistent iron absorption control. Lower Css min value in the treatment 1 compared with that in the treatment 1 showed that iron absorption was better in the treatment 2, that was, 2 hours after meal.

CONCLUSION

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

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