The effects of weekly iron supplementation with folic acid, vitamin A, vitamin C on iron status of Indonesian adolescents

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The goal of the study was to determine whether the addition of vitamins A and C and extra folate to the commonly distributed iron-folate supplement administered on a weekly basis would result in improved iron status in anaemic and non-anaemic female adolescents. Subjects (n=84) were selected if their haemoglobin concentration in venous blood was ≤140g/L. Subjects were randomly allocated into 2 groups. One group received 60mg iron, 500µg folic acid, 20 000 IU vitamin A and 60mg vitamin C weekly, while the other group received 60 mg iron and 250µg folic acid weekly for a period of 5 weeks. Before and after treatment, haemoglobin, serum ferritin and body height and weight were determined. All subjects received anthelmintic drugs before supplementation. A complete data set was obtained from 42 young women in the group supplemented with iron, folic acid, vitamin A and vitamin C and from 40 young women in the group supplemented with iron and folic acid. Haemoglobin, mean cell volume and serum ferritin increased significantly in both groups (P<0.05). Among anaemic adolescents, the rise in haemoglobin concentration in the multisupplement group was higher than that in the iron folate group. The result confirms that weekly supplementation is effective in improving iron status in a short time (5 weeks) and that supplementation with vitamins as well as iron results in an additional improvement in haemoglobin concentration.

Key words: Anaemia, iron supplementation, vitamin A, adolescents, Indonesia

Introduction

It is well documented that iron deficiency anaemia is an important public health problem in most developing countries[1]. The most affected groups are pregnant women and children. As a result, since the 1970's, iron supplementation programs have been established in many countries. However, the programs have not succeeded in reducing, significantly, anaemia. Factors such as low coverage rates of the public health care system and insufficient tablet distribution contribute to reduced efficiency of supplemental iron programs[2]. Another major constraining factor is low compliance due to negative side effects of the tablets or the fact that women are inadequately informed about the reason for administration of iron supplements[3].

New dosing regimens of iron supplementation may help improve the efficiency of iron supplementation programs. Wright and Southo[4] demonstrated that giving anaemic rats iron every second day has a similar effect on the improvement of the iron status as daily administration of iron. At the end of the study rats given iron daily had received 133% more iron than those given iron every other day. Therefore less frequent supplementation with a smaller amount of iron was suggested for humans. Several studies have shown that the frequency of supplementation can be reduced without reducing the efficacy of the supplementation. Schultink et al[5] found that a biweekly iron supplementation in preschool children in Indonesia resulted in a similar increase in haematological values as a daily supplementation. According to the study of Gross et al[6], supplementation on a weekly basis with a relatively low dose of medicinal iron is as effective in improving iron status as daily supplementation.

Mejia and Chew[7] found that the addition of vitamin A to iron supplements improves haematological status and iron metabolism in anaemic children. An additional benefit of a combined iron-vitamin A supplementation program in comparison with iron supplementation alone has also been shown in pregnant women[8]. These studies were based on daily iron supplementation schedules, and it has not yet been investigated whether an iron-vitamin A supplementation on a weekly basis would be equally effective.

Other micronutrients considered important in improving iron status are folic acid and vitamin C[9,10]. In the current Indonesian iron supplementation program for pregnant women, folic acid is included in the tablets because folate deficiency often accompanies iron deficiency and may lead to anaemia. Vitamin C plays an important role in improving the absorption of iron in the gut. The addition of vitamin C to an oral iron supplement, such as ferrous sulphate, improves absorption.

Until now iron supplementation programs have targeted

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pregnant women with a curative approach. However, the depletion of iron stores in women starts during adolescence with the onset of menstruation. Therefore, iron supplementation programs with a preventive approach may have the advantage of building up and maintaining the iron stores before pregnancy[11].

The purpose of this study was to investigate whether a supplement containing iron and folic acid, vitamin A and vitamin C would be more effective than a supplement containing only iron and a smaller amount of folic acid. Furthermore, the effectiveness of weekly iron supplementation to improve iron status of young women was studied.

Subjects and methods

The study area was located in a middle socio-economic class area in Jakarta, Indonesia. The district has a population of circa 280 000 people and a population density of 26 000 persons per square km[12].

Subjects

805 girls were enrolled in a baseline survey to investigate the prevalence of anaemia. The selection criteria for the supplementation study were regular school attendance, age 13-16 years, already menstruating, and a haemoglobin concentration <120 g/L, because anaemia is likely to be present in these individuals[13]. Because only 24 girls were eligible by these criteria, 60 additional girls who met the criteria but had a haemoglobin concentration between 120 and 140g/L were randomly selected to be included in the study. Stool samples of 37 subjects were examined for the presence of parasitic infections (ascariasis and trichuriasis). Because 30% of these girls were infected, all 84 girls received an anthelmintic treatment before the supplementation period[14].

The study was carried out for 5 weeks in April and May 1995. The international ethical guidelines for epidemiological studies were followed in this study[15]. The Ethical Review Committee of the University of Indonesia, Jakarta, approved the research proposal. The parents of the girls gave their consent.

Methods

The 84 girls were assigned at random to two groups. Group 1 (n=43) received weekly supplements containing 60 mg elemental iron, 500μg folic acid, 20 000 IU vitamin A in the form of retinol acetate, and 60mg ascorbic acid for a period of 5 weeks. Group 2 (n=41) received, during the same period weekly supplements containing only 60mg elemental iron and 250μg folic acid. At the beginning of each week, the girls received one tablet. The appearances of the tablets were different. The tablet containing iron, folic acid, vitamin A, and vitamin C was orange and the tablet containing iron and folic acid was red. The supplements were produced by Pt Kimia Farma, Jakarta, Indonesia. The supply of the tablets was distributed and closely supervised at the school by two nutritionists and the first author.

Before and after the supplementation, body height and weight were measured. Body weight was measured with an electronic scale (SECA 770 alpha; SECA; Hamburg), and stature was measured by using a microtoise.

At the beginning and end of the study, a medical technician took 5mL venous blood from the girls for the haematological analyses. These analyses were determined using Coulter MAXM 1000 (Coulter Corporation, Miami, USA) at the Department of Clinical Pathology, Medical Faculty, University of Indonesia. The haemoglobin concentration was measured by the cyanmethaemoglobin method[16]. Serum Ferritin was analysed according to the enzyme linked immunoassay (ELISA) "Spectro Ferritin" from Ramco Laboratories Inc, Houston[17].

Additionally a structured interview was taken with a questionnaire regarding compliance with the supplementation regimen and food habits.

Statistical methods

The statistical analysis was carried out using SPSS for Windows, Version 6.0[18]. Within each group the change in haemoglobin and mean cell volume after supplementation was investigated by using paired t-test ($P<0.05$). Because serum ferritin concentration was not normally distributed changes after supplementation were evaluated by using Wilcoxon matched pairs signed-ranks test. Furthermore, three way repeated measures analysis of variance (ANOVA) was used to test the influence of time, dosing and degree of iron status on the effectiveness of supplementation. Correlations between initial haemoglobin and changes in haemoglobin, mean cell volume and serum ferritin were calculated.

Results

As shown in Table 1, the final data set contained 42 subjects in Group 1 (iron, folic acid, vitamin A and vitamin C) and 40 subjects in Group 2 (iron and folic acid). Two young women did not complete the study, one became ill and the other did not return for the second blood sampling.

Table 1. Selected characteristics of subjects at the beginning of the study[a,b].

	Group 1[d] (n = 42)	Group 2[e] (n = 40)
Weight (kg)	46.3±7.61	45.6±6.1
Height (cm)	153.1±4.5	153.3±4.4
Age (years)	14.6±0.9	14.5±0.6
BMI[c]	19.7±2.7	19.4±2.1

a. Mean±SD. Group 1 was supplemented weekly with Fe + Folic Acid + Vit A + Vit C and Group 2 with Fe + Folic Acid
b. There was no significant difference between the groups
c. Body Mass Index
d. Fe + Folic Acid + Vit A + Vit C
e. Fe + Folic Acid

At the beginning of the study there were no significant differences in weight, height, age and body mass index between the two groups. Furthermore, there were also no significant differences in any of the haematological values (Table 2).

According to Table 2, at the end of the supplementation period the mean values of serum ferritin of both groups had increased significantly (Group 1: $P=0.022$; Group 2: $P<0.001$). However, the haemoglobin concentration and MCV had increased significantly only in the group given the multinutrient supplement (Hb: $P=0.045$; MCV: $P=0.013$). The haemoglobin concentration increased significantly in the two subsets of anaemic girls (Group 1: P=0.011; Group 2: P=0.018). On the other hand, serum ferritin increased significantly only in the two subsets of non-anaemic girls (Group 1: P=0.013; Group 2: P=0.002). The haemoglobin concentration of two anaemic girls of Group 1 and one anaemic girl in Group 2 increased above the cut-off point of 120g/L.

Table 2. Haematological values before and after 5 weeks supplementation[a].

	Before	After	Difference
Group 1: Fe ± Folic Acid ± Vit A ± Vit C (n=42)			
Haemoglobin (g/L)	122±12	124±11	1.8±0.6b
Mean cell volume (fL)	81.6±6.7	82.1±6.2	0.54±1.35b
Serum ferritin (μg/L)	26.4±20.4	40.0±29.1	13.6±23.5f
Subgroup anaemic (n=11)			
Haemoglobin (g/L)	106±13	112±15	6.2±6.6b
Mean cell volume (fL)	75.7±7.3	76.6±6.4	0.9±1.6
Serum ferritin (μg/L)	15.2±14.9	21.7±22.6	6.5±17.5
Subgroup non-anaemic (n=31)			
Haemoglobin (g/L)	128±4	128±6	0.2±4.3
Mean cell volume (fL)	83.6±5.2	84.0±4.8	0.4±1.2
Serum ferritin (μg/L)	30.4±20.7	46.6±28.6	16.2±25.0e
Group 2: Fe ± Folic Acid (n=40)			
Haemoglobin (g/L)	121±14	121±13	0.8±5.4
Mean cell volume (fL)	81.6±10	82.0±9.6	0.39±1.5
Serum ferritin (μg/L)	30.1±22.8	43.9±35.9	13.8±30.1e
Subgroup anaemic (n=13)			
Haemoglobin (g/L)	106±17	110±16	4.0±5.3b
Mean cell volume (fL)	72.5±11	73.7±11.3	1.2±0.9c
Serum ferritin (μg/L)	14.8±16.5	20.3±21.8	5.6±21.1
Subgroup non-anaemic (n=27)			
Haemoglobin (g/L)	127±5	126±6	-0.8±4.8
Mean cell volume (fL)	85.9±5.9	85.9±5.5	0.01±1.5
Serum ferritin (μg/L)	37.5±21.9	55.2±36.0	17.8±6.4d

a. Mean ± SD
b. Before vs after supplementation, P<0.05 (paired t-test)
c. Before vs after supplementation, P<0.001 (paired t-test)
d. Before vs after supplementation, P<0.05 (Wilcoxon test)
e. Before vs after supplementation, P<0.01 (Wilcoxon test)
f. Before vs after supplementation, P<0.001 (Wilcoxon test)

A significant correlation (P<0.001) was found between initial haemoglobin concentration and changes in haemoglobin (r=0.90) and mean cell volume (r=0.73). Since more girls with a low iron status had been randomly allocated to Group 1 than to Group 2, the initial haemoglobin concentration was considered statistically as an additional factor in the analysis of variance. The initial haemoglobin concentrations were divided into three classes: <100g/L (n=5); 100-120g/L (n=19); >120g/L (n=58).

There was a significant effect of treatment type for haemoglobin (P=0.003) and mean cell volume (P=0.02), but not for serum ferritin (P=0.82) (Table 3). After 5 weeks there was no significant effect of time for these 3 variables although the effect on haemoglobin was close to the 95% level (P=0.068). For haemoglobin there was a significant interaction (P=0.041) between treatment type and haemoglobin class. There was no significant interaction between treatment effect and treatment type for haemoglobin (P=0.73), mean cell volume (P=0.98) or serum ferritin (P=0.89) (Table 3).

There were no significant changes in weight or height after the supplementation.

The results of the questionnaire showed that 70.8% of the girls took all 5 tablets, 25.6% took 4 tablets and 3.6% ingested 3 tablets. All girls consumed rice more than once a day. More than 90% of them ate animal protein, such as meat, eggs or chicken, more than once a week. About 30% of the girls consumed fruits such as papaya, banana, and pineapple more than 3 times a week.

Table 3. Analysis of variance.

Independent factors	Dependent factors (P values)		
	Haemoglobin	Mean Cell Volume	Serum Ferritin
Within subjects			
Treatment effect (time)	0.068	0.68	0.39
Between subjects			
Treatment type (Fe+Folic Acid + VitA + VitC vs Fe+Folic Acid)	0.003	0.02	0.82
Initial haemoglobin (three classes)	0.00	0.00	0.17
Interaction			
Treatment type x haemoglobin class	0.04	0.27	0.92
Treatment effect x type	0.73	0.98	0.89
Treatment effect x haemoglobin class	0.55	0.95	0.95
Treatment effect x type x haemoglobin class	0.73	0.89	0.72

Discussion

According to Tables 2 and 3, iron supplementation on a weekly basis for 5 weeks resulted in an improvement of the iron status in adolescence females. This result confirms earlier observations about the effectiveness of weekly iron supplementation[5,6]. In the anaemic sub groups the improvement was an increase in the haemoglobin concentrations whereas in the non-anaemic groups the improvement was an increase in the serum ferritin levels (Table 2). These data support the conclusion that in anaemic persons the absorbed iron is at first utilised to normalise the haemoglobin concentration in the plasma for its essential functional role to supply the tissue with oxygen. Only when the haemoglobin concentration is close to a satisfactory level will iron stores be replenished by increasing the serum ferritin levels[19]. Therefore the five dosages were not enough to normalise the iron status in the anaemic adolescents.

The results give a less clear answer whether a positive effect can be observed by the addition of vitamin A and vitamin C to the supplementation tablets. An "eye-ball analysis" of the results in the Table 2 suggests that all iron states improved more with the addition of the two vitamins compared to the traditional iron-folate tablet. However, contrary to the results described by Mejia and Chew[7] the analysis of variance did not show a clear beneficial effect of vitamin A on the iron status because there was no significant interaction between treatment effect and the type of treatment and the significant main effect of treatment type for both haemoglobin and mean cell volume does not consider the time effect of the treatment. This result may be explained by the fact that 5 weeks was too short to show a significant difference.

There was a significant effect of supplementation on serum ferritin in both groups as a whole and in both sub groups of nonanaemic girls (Table 2). This is likely because 70% of the girls had an adequate iron status and supplementation increased iron storage as previously mentioned. The lack of a clearly observable effect of vitamins A and C on ferritin level in the ANOVA might be due to adequate consumption of both vitamins in this middle income group.

The supplementation period (5 weeks) was too short to expect all anaemic individuals to become non-anaemic. Bothwell et al[20] reported that the rate of haemoglobin synthesis can increase as the haemoglobin levels of the anaemic individuals increase such that the mean level is 2/3 of the way between the initial level and the non-anaemic cut-off level.

However, this has only been investigated with a daily supplementation regimen and not yet with supplementation on a weekly basis. Therefore, it should be tested whether a higher dosage of iron in the tablet administered on a weekly basis could shorten the time required to increase haemoglobin concentration to non-anaemic levels.

However, one must consider that iron absorption is inversely related to the amount of iron present in the duodenum-jejunum, whereas the frequency of gastrointestinal side-effects is directly proportional to the amount of iron present. With a low dose of medicinal iron only a small amount of iron comes in contact with the duodenal mucosa at any one time. This improves absorption and gastrointestinal tolerance, and consequently compliance increases because of fewer side-effects[1].

During the five weeks, only 70% of the adolescents took all five tablets. The reason for the less than 100% compliance was the absenteeism of the surveyed adolescents during the five weeks of the study. Thirty percent of the adolescents reported nausea, headache, or sleepiness. Studies among pregnant women in urban Jakarta[5] and rural Sulawesi[21] estimated compliance with prescribed tablet intake to be as low as 33% which may be due to the side-effects caused by daily intake of iron tablets. In addition, some women did not see the necessity of taking the tablets and some women forget to take the tablets. Compared to these studies, the compliance among the adolescents was far higher. However, in this study the intake of tablets was supervised.

This study confirms the effectiveness of iron supplementation in adolescent girls even after ingestion of only five tablets on a weekly basis. An additional effect of vitamins A and C could not be clearly observed which needs to be studied in low income groups where reduced vitamin A intakes can be expected over a longer period of time. Further investigations are necessary to explore whether a longer period of supplementation and/or a higher dosage of iron with or without a higher dosage of folic acid, and vitamins A and C are necessary to reduce the prevalence of anaemia in adolescents.

When a national strategy is developed for the alleviation of poverty and the development of a health care system, specific micronutrient programs should be promoted. When the promotional efforts have achieved agreement among political leaders and the public, an action plan can begin with four simultaneous programs, each with a progressively longer-term goal:

(1) highly targeted, rapid interventions through the delivery of vitamin and mineral pills and other pharmaceutics;
(2) longer-term interventions through fortification of selected foods, if feasible;
(3) consumer education programs to modify diets by building awareness of micronutrients; and
(4) coordinated agricultural programs to increase the supply of micronutrient-rich food[11].

Schools or factories, with support from the health care system, could be addition sites for assessing iron status, delivering iron tablets and supervising tablet intake. The iron status of young women would be improved before pregnancy and as a consequence the risk of mortality and morbidity for mother and fetus would decrease.

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每周補充鐵和葉酸、維生素 A 、維生素 C 對印尼青少年鐵營養狀況的影響

摘要

該研究目的是測定每周補充維生素 A 、 C 和葉酸是否會改善貧血和非貧血女青少年的鐵營養狀況。作者選擇了 84 位靜脈血液血紅蛋白爲 ≤ 140 克/升的女青少年爲對象，隨機分成兩組，一組每周給予 60 毫克鐵、 500 微克葉酸、 20,000 IU 維生素 A 和 60 毫克維生素 C ，另一組給予 60 毫克鐵和 250 微克葉酸。共給予 5 周，治療前後測定了血紅蛋白、鐵蛋白、體重和身高。補充前所有對象給予驅虫劑治療。結果顯示，兩組的血紅蛋白、平均血細胞體積和血清鐵蛋白均明顯增加（ $p<0.05$ ）。在貧血女青少年中，補充多種維生素組血紅蛋白濃度升高，較單單補充鐵和葉酸組爲大。這個結果証實了短期 5 周的補充可改善鐵營養狀況，同時補充維生素和鐵可升高血紅蛋白的濃度。

References

1. DeMaeyer E, Dallman PR, Gurney JM, Hallberg L, Sood SK, Srikantia SG. Preventing and controlling iron deficiency anaemia through primary health care. Geneva: World Health Organization, 1992.
2. ACC/SCN. Controlling Iron Deficiency. ACC/SCN State-of-the-art-series. Nutrition policy discussion paper No.9. Geneva: ACC/SCN, 1991.
3. Galloway R. Determinants of Medical Compliance. Washington DC: The World Bank, 1990.
4. Wright AJA, Southo S. The effectiveness of various iron supplementation regimes improving the Fe status of anaemic rats. Br J Nutr 1990; 63: 579-58.
5. Schultink W, Gross R, Gliwitzki M, Karyadi D, Matulessi P. Effect of daily vs twice weekly iron supplementation in Indonesian preschool children with low iron status. Am J Clin Nutr 1995; 61: 111-115.
6. Gross R, Schultink W, Juliawati. Treatment of anemia with weekly iron supplementation. Lancet 1994; 8925: 821.
7. Mejia LA, Chew F. Hematological effect of supplementing anaemic children with vitamin A alone and in combination with iron. Am J Clin Nutr 1988; 48: 595-600.
8. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JGAJ. Supplementation with vitamin A and iron for nutritional anemia in pregnant women in West Java, Indonesia. Lancet 1993; 342: 1325-1328.
9. International Nutritional Anemia Consultative Group (INACG). Iron Deficiency in women. Washington DC: Nutritional Foundation, 1984.
10. International Nutritional Anemia Consultative Group (INACG). Combating Iron Deficiency in Chile: A case study. Washington DC: Nutrition Foundation, 1986.
11. World Bank. Enriching Lives. Washington DC: World Bank, 1994.
12. Kantor Statistik. Penduduk wilayah kotamady Jakarta Timur 1990. Hasil Sensus Penduduk. Jakarta, 1991.
13. WHO. Technical report series No. 405; Nutritional anemias: report of a WHO scientific group. Geneva: World Health Organization, 1968.
14. WHO. The treatment and management of severe protein-energy malnutrition. Geneva: World Health Organization, 1981.
15. CIOMS. International Guidelines for Ethical Review of Epidemiological Studies. Geneva: Council for International Organizations of Medical Sciences, 1991.
16. International Nutritional Anemia Consultative Group (INACG). The design and analysis of iron supplementation trials. Washington DC: Nutritional Foundation. 1984.
17. Manual "Spectro Ferritin", an enzyme immunoassay procedure for the quantitative analysis of serum ferritin. Houston, Texas: Ramco Laboratories Inc., 1987.
18. Norusis MJ. SPSS for Windows. Release 6.0. Chicago: SPSS Inc., 1993.
19. Dallman PR. Iron. In: Present Knowledge of Nutrition. Sixth Edition, ed ML Brown. Washington DC: Nutrition Foundation, International Life Sciences Institute, 1990, pp 241-249.
20. Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron metabolism in man. Oxford: Blackwell, 1979.
21. Thorand B, Schultink W, Gross R, Sastroamidjojo S, Wentzel S. Efficiency of the iron supplementation program for pregnant women in Jeneponto, South Sulawesi, Indonesia. Asian Pacific J Clin Nutr 1994; 3: 211-215.