

Effect of Daily and Intermittent Iron Supplementation on Iron Status of High School Girls

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Abstract: This experimental study was designed to investigate the effects of daily versus intermittent iron supplementation on iron status of high school girls in Zahedan and Rasht cities in 1996–1997. The subjects were selected randomly from among students of grades 1–3 of four high schools in each city. Anemia was determined by measuring hematological indices. 260 anemic and a similar number of non-anemic subjects of 4 high schools were selected and allocated randomly to 4 treatment groups. During a 3-month period, the test groups were given 150 mg ferrous sulfate tablets (50 mg Fe). Subjects in group 1 received a daily dose, groups 2 & 3 received twice or once weekly doses respectively. The control group received no iron supplement. For these subjects, in addition to hematological indices biochemical iron indices were measured in the beginning and at the end of the study. The increases in hemoglobin concentration in anemic subjects were not significantly different among supplemented groups but were different from the control group ($p < 0.00001$). Among anemic subjects, changes in serum ferritin levels in 3 supplemented groups were significantly different from the control group. Serum ferritin in Group 1 was also increased to a greater extent than groups 2 and 3 ($P < 0.00001$). It is concluded that over the study period a weekly iron dose was as effective as a daily dose in treating anemia but the daily dose was more effective in improving iron stores than a weekly dose in the short run.

Key words: Iron deficiency anemia, iron status, iron supplementation, adolescent girls

Introduction

Iron deficiency anemia is one of the most important nutritional and public health problems in the world with serious consequences [1–8]. According to WHO, on a global basis, over 2000 million people especially children and

women of child-bearing age in developing countries are affected by anemia or iron deficiency [2]. National reports on anemia in Iran showed prevalence of anemia, iron deficiency anemia and iron deficiency among 15 to 49-year old females to be 31%, 14.5% and 39% respectively [9].

Oral iron supplementation, fortification, diet modification and health services are the most important approaches to combating iron deficiency anemia recommended by international organizations [1, 4, 10]. Iron supplementation programs have been accepted as a useful, targeted and cost-effective measure to control iron deficiency but compliance is known to be poor. Programs are not working perfectly due to several factors including low efficacy of

Abbreviation used: Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cell count; SI, serum iron; TIBC, total iron-binding capacity; TS, transferrin saturation; SF, serum ferritin; BMI, body mass index; A, anemic; NA, non anemic.

health care system and the side effects of supplements [1, 4, 11–13].

Although the results of recent human studies and laboratory animals have shown the effectiveness of weekly iron supplementation in prevention and control of anemia [1, 3, 11–21], WHO and other international organizations have recommended further clinical trials in different countries and age groups especially adolescent girls as a vulnerable group and would-be mothers [1, 2, 10, 22].

The aim of present study was, therefore, to examine the effects of daily and intermittent iron supplementation on hematological and biochemical iron indices in high school girls in Zahedan & Rasht cities in Iran in 1996–1997 during a 3-month period.

Materials and Methods

Subjects: Altogether 1853 subjects (945 in Zahedan & 908 in Rasht) were selected from among students of grades 1–3 in four high schools, by stepwise random sampling from among all public high schools, in each city. Anemia in subjects was determined at baseline by hemoglobin concentration adjusted for altitude adding a correction factor of 4% for each 1000 meters above sea level [23]. Therefore anemia status criteria in Rasht (sea level) was taken as Hb levels below 12 g/dl, while in Zahedan (an altitude of 1370 meters from sea level) 12.7 g/dl [2, 24]. Mentzer formula ($MCV/RBC < 13$) was used to identify and exclude suspected thalassemic cases [25, 26]. The anemic (A) and an equal number of non-anemic (NA) subjects in the 4 high schools were chosen by these criteria and were considered as to participate in the intervention phase and were randomly allocated to 4 treatment (1, 2, 3 & control) groups (Table I). Written consent was secured from all subjects families. The study protocol and ethical aspects was approved by research council of National Nutrition Research Institute.

Samples characteristics: Data on demography, menstruation, illness history including surgery, severe bleeding, hemorrhoid, hemophilia and parasitic infections, medica-

tions, vitamin and mineral supplementation were collected by interviews. Weight and height were measured by using Soehnle digital scale and measuring tape with minimal clothing. Food consumption pattern was evaluated by a 24-hour recall questionnaire for three consecutive days in the second month of the study. Energy and nutrients, protein, vitamin C and iron, intake was calculated by using Food Processor software program modified by incorporating Iranian Food Composition Table.

Intervention: The three-month intervention phase was carried out in Winter 1996 and Spring 1997 in the urban areas of Zahedan and Rasht (capitals of Sistan-Baluchestan and Gilan provinces in Iran). The test groups were given 150 mg ferrous sulfate tablets containing 50 mg elemental iron, at least one hour after dinner & before sleeping to be taken with water. Therefore subjects in group 1, received a daily dose of iron (7 days/wk), group 2 & 3 received an intermittent dose, twice weekly (Saturdays & Wednesdays) and once weekly (Wednesdays) respectively. The control group received no iron supplement (Table I). The average weekly iron intake, thus, was 350, 100 & 50 mg in groups 1, 2 & 3 respectively. Compliance with taking tablets was calculated by counting the remaining tablets. Specific side effects of iron supplements were investigated by asking the girls at the second and third months of study period.

Hematological measurements: 10 ml nonfasting venous blood samples were collected from all subjects at baseline and only from subjects participating in intervention phase after 3 months of supplementation. Blood was collected in 2 tubes, one tube containing EDTA for the analysis of hematological indices including Hb, Hct, MCV, MCH, MCHC, RBC (2 ml blood) and the second one for biochemical iron indices including SI, TIBC, SF (8 ml blood). Blood was transported to the laboratory in a cool box. Hematological indices were measured using automatic cell counters within 5 hours after blood collection. Blood serum was obtained by centrifugation for 10 min at $3000 \times g$ at 25°C temperature and was stored at –20°C before being transported to Tehran under freezing condition until analyzed. SI and TIBC were measured manually employ-

Table I: Number of subjects in each group

city	group 1		group 2		group 3		control		total	
	A	NA	A	NA	A	NA	A	NA	A	NA
Zahedan	37	41	42	46	58	59	47	49	184	195
Rasht	8	6	11	13	30	24	27	25	76	68
Both	45	47	53	59	88	83	74	74	260	263

A = Anemic

NA = Non-Anemic

Table II: Selected characteristic of subjects at the beginning of the study

	Anemic (n = 260)	Non-Anemic (n = 263)
Characteristics	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Age (year)	16.3 $\pm$ 1.3	16.3 $\pm$ 3.6
Family size	7.4 $\pm$ 2.3	7.7 $\pm$ 2.5
First menstruation (year)	13.3 $\pm$ 1.1	12.9 $\pm$ 2.4
Menses duration (day)	6 $\pm$ 1.2	5.8 $\pm$ 1.2
Weight (kg)	48.1 $\pm$ 7.7	49 $\pm$ 8.9
Height (cm)	157.2 $\pm$ 5.8	156.7 $\pm$ 5.6
BMI (kg/m ²)	19.4 $\pm$ 2.8	19.9 $\pm$ 3.2
Food consumption pattern	(n = 187)*	(n = 148)*
Energy (kcal)	1558.8 $\pm$ 499.5	1579.7 $\pm$ 531.2
Protein (gr)	48.7 $\pm$ 17.8	51.2 $\pm$ 19.5
Vitamin C (mg)	36.1 $\pm$ 24.3	43.6 $\pm$ 33.6
Iron (mg)	18 $\pm$ 7.9	17.9 $\pm$ 6.9

There were no significant differences in these variables between anemic & non-anemic subjects.

* Food consumption pattern was evaluated for a subsample of anemic & non-anemic subjects.

ing Zist Chimi company kits (LotCo 31 & LotLo 57) and colorimetric method. TS was calculated using the formula of $(SI/TIBC) \times 100$. SF was measured using Iran Kavoshyar kit (Code 3000) and immunoradiometric assay (IRMA) with an automatic gamma-counter (Packard model).

Statistics: Independent and paired t-tests, one way Analysis of Variance (ANOVA) with the Scheffe's multiple comparison test, McNemar test and SPSS/PC software were used for statistical data sets analysis. The data are presented as means and standard deviations.

Results

Prevalence of anemia was 23% and 11.6% in Zahedan and Rasht cities respectively with a combined prevalence of 17.4%. Some features of anemic and non-anemic subjects

at the beginning of the study are shown in Table II. The prevalence of iron deficiency (SF < 12 ng/ml) in A & NA subjects was 56.4% (Table III).

In Table IV baseline hematological and biochemical iron indices in anemic and non-anemic subjects are shown for Zahedan and Rasht cities. No significant differences were observed in age, duration and age of first menstruation, weight, height, BMI, energy and nutrient intakes as well as other intervening factors among the four treatment groups. Nor were there any significant differences in initial hemoglobin and ferritin concentrations (Table V). After 3 months of supplementation, there were significant "within-group" increases in Hb and SF concentrations in weekly and daily supplemented groups ($p < 0.007$). Mean changes in Hb concentration in anemic subjects during the study were not significantly different among 3 supplemented groups but were significantly different from the

Table IV: Hematological & biochemical iron indices in anemic & non-anemic subjects in Zahedan and Rasht at baseline

	Anemic	Non-Anemic
	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Indices	(n)	(n)
<i>Zahedan</i>		
Hb (gr/dl)	11.6 $\pm$ 1 (184)	13.7 $\pm$ 0.6* (195)
Hct (%)	36.8 $\pm$ 2.8 (184)	42 $\pm$ 1.8* (195)
TS (%)	13.2 $\pm$ 8.6 (144)	18.9 $\pm$ 8.1* (121)
SF (ng/ml)	9.4 $\pm$ 13.3 (178)	18.1 $\pm$ 17.4* (192)
<i>Rasht</i>		
Hb (gr/dl)	11.2 $\pm$ 0.6 (76)	13.6 $\pm$ 0.9* (68)
Hct (%)	37 $\pm$ 2.4 (76)	41.5 $\pm$ 2.9* (68)
TS (%)	18.3 $\pm$ 8.6 (65)	18.6 $\pm$ 8.3 (59)
SF (ng/ml)	13.3 $\pm$ 14.9 (73)	16.8 $\pm$ 9.8 (66)

* Significantly different from anemic subjects ($p < 0.0001$)

Table III: Serum ferritin (SF) distribution of the subjects at baseline

SF	Anemic		Non-anemic		Total	
	No.	%	No.	%	No.	%
SF < 12 ng/ml (iron deficiency)	174	69.3	113	43.8	287	56.4
12 $\leq$ SF < 25 ng/ml (low iron stores)	48	19.1	95	36.8	143	28.1
SF $\geq$ 25 ng/ml (reasonable iron stores)	29	11.6	50	19.4	79	15.5
Total	251	100	258	100	509	100

Table V: Concentration of hemoglobin & ferritin in anemic and non-anemic subjects at baseline and the changes after 3 months of supplementation compared to baseline

	group 1 $\bar{X} \pm SD$ (n)	group 2 $\bar{X} \pm SD$ (n)	group 3 $\bar{X} \pm SD$ (n)	control $\bar{X} \pm SD$ (n)
<i>Baseline</i>				
A	Hb (gr/dl)	11.7 $\pm$ 0.7 (45) a	11.6 $\pm$ 1 (53) a	11.4 $\pm$ 1 (88) a
	SF (ng/ml)	9.6 $\pm$ 10.5 (43) a	10.8 $\pm$ 15.5 (52) a	11.6 $\pm$ 15.6 (85) a
NA	Hb (gr/dl)	13.7 $\pm$ 0.6 (47) a	13.8 $\pm$ 0.8 (59) a	13.5 $\pm$ 0.7 (83) a
	SF (ng/ml)	18.4 $\pm$ 17.8 (45) a	14 $\pm$ 8.7 (56) a	18.9 $\pm$ 19.6 (83) a
<i>Differences</i>				
A	Hb (gr /dl)	1.2 $\pm$ 1 (45) a	1.2 $\pm$ 1.1 (53) a	1.3 $\pm$ 1.1 (88) a
	SF (ng/ml)	21.7 $\pm$ 19.2 (42) a	8.9 $\pm$ 19.9 (52) b	9 $\pm$ 19.1 (81) b
NA	Hb (gr/dl)	0.3 $\pm$ 0.7 (47) a	0.3 $\pm$ 0.7 (59) b	0.6 $\pm$ 0.8 (83) b
	SF (ng/ml)	23 $\pm$ 17.9 (43) a	15.5 $\pm$ 15.4 (54) b	11.7 $\pm$ 18.2 (80) b

In each row, the mean values with different letters are significantly different

1 = $p < 0.0004$ and $p < 0.00001$ for all other differences

control group ($p < 0.00001$, Table V). In spite of lower hematologic response to iron supplements in comparison with anemic subjects, administration of daily and weekly iron doses were similarly effective on Hb changes in non-anemic subjects (Table V).

Among anemic and non-anemic subjects changes in serum ferritin levels in 3 supplemented groups were significantly different from the control group. Serum ferritin in group 1 was also increased to a greater extent than groups 2 and 3 ($p < 0.00001$, Table V).

After 3 months of supplementation, the prevalence of anemia in groups 1, 2 and 3 decreased by 35%, 35% and 29% ($p < 0.0005$). The prevalence of iron deficiency (SF < 12 ng/ml) in anemic as well as non-anemic subjects decreased by 56.5%, 26% and 24% in 3 supplemented groups respectively ($p < 0.0005$).

Compliance in anemic and non-anemic subjects receiving iron supplements was 70 to 90 percent on the average. No significant differences in the incidence of side effects were observed among daily and twice as well as once weekly supplemented groups. Also no one was omitted because of serious side effects of supplements during the study.

Discussion

Our data show the hemoglobin and serum ferritin levels to have improved significantly in the supplemented groups

compared to the control subjects. Iron deficiency anemia as a problem appears in 3 stages. In the first stage, lack of iron leads to depletion of iron stores followed by a drop in serum ferritin concentration. Under such conditions SF levels below 12 ng/ml and between 12 and 25 ng/ml are defined as "iron deficiency" and "low iron stores" respectively. In the second stage (iron deficiency for erythropoiesis), TS is reduced to below 16% while during the third stage the concentrations of Hb and other RBC indices fall below the standard levels [27, 28]. Comparison between the mean iron status indices in anemic and non-anemic subjects (Table IV) with standard criteria showed firstly that anemia is due to iron deficiency and low iron stores and secondly iron deficiency was also present in non-anemic subjects. The mean concentrations of TS & SF in NA subjects points to probable existence of first and second stages of iron deficiency anemia. Thus it can be concluded that these girls will be at a higher risk of anemia in the future, necessitating universal supplementation programs without prior screening.

There were no significant differences in initial values of all indices among 4 groups (Table V), thus mean changes of indices were discussed. Because of no significant differences in intervening factors including parasitic infection, ... among 4 study groups, we can attribute these changes to 3-month administration of iron supplements. Intermittent (twice and once weekly) iron supplementation was as effective as daily iron supplementation in improving mild anemia in spite of the fact that total iron intake in twice and once weekly supplemented groups were

2/7 & 1/7 of iron intake in daily supplemented group respectively. These results are in accordance with other studies conducted in preschool children, pregnant women and adolescent girls [3, 11, 12, 16, 17]. Supplemental iron reaching mucosa less frequently during the turn over period (5 to 6 days in human) will result in higher absorption efficacy, while, daily iron administration will be less efficient and more wasteful. Intermittent dosing would also result in less iron overload due to unassimilated iron, which may be related to undesirable side effects reported for daily supplements [3, 11, 14, 21, 22].

Regarding changes in SF after 3 months of supplementation, both daily and intermittent doses could produce a significant increase in body iron stores but daily dose was more effective so. Previous studies have pointed to progressive and sustained improvement in iron stores with less frequent dosing whereas daily doses are not as efficacious as weekly doses due to gradual decrease in iron absorption. In studies which have evaluated long term effects of these two approaches, the effect of daily and weekly supplementation was similar 4 to 6 months after discontinuation of supplements. Significant differences in final SF levels between daily and weekly supplemented groups disappeared shortly after supplementation [12, 22].

WHO is in the position that in large scale public health programs particularly in developing countries, where systematic laboratory testing is organizationally and financially impossible, the best approach to control and prevention of anemia & iron deficiency is to give iron supplements to the entire high risk groups [2, 10, 17, 22].

In view of high prevalence of iron deficiency among adolescent girls, universal weekly iron supplementation which is simple, cost-effective and safe, can be recommended as a key strategy for controlling iron deficiency.

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