Effect of iron supplementation on biochemical indices of iron status in selected pre-adolescent schoolgirls in North West Frontier Province, Pakistan

Parvez I. Paracha MS, PhD*, S.M. Khan MBBS, MSc, MRCP, I. Ahmad MSc, PhD and G. Nawab MSc, MPhil†

**Department of Human Nutrition, NWFP Agricultural University, Peshawar, Pakistan; †Institute of Radiotherapy and Nuclear Medicine, Peshawar University, Peshawar, Pakistan.*

A study was carried out on eight to 11 year old schoolgirls to assess the prevalence of iron deficiency anemia (IDA) and to study the impact of iron supplementation on the biochemical indices of iron status. The children were characterized iron deficient anemic if their serum ferritin levels (SF) were $\leq$ 12 ng/ml and hemoglobin (Hb) $<$ 12 g/dl or hematocrit (Hct) $\leq$ 35%. In a double blind trial, the anemic and non-anemic children were randomly selected for the treatment and control groups. All the groups received multivitamin tablets daily, the treatment group received an additional 76 mg elemental iron per day for 11 weeks. The prevalence of IDA in these children was found to be 35%. The supplementation caused a significantly ($P<0.05$) greater change in SF (20 ng/ml); Hb (1.5 g/dl) and Hct (3%) of the anemic treatment group compared to the corresponding control group. The non-anemic treatment group also showed a significantly greater change in SF (9 ng/ml); Hb (0.78 g/dl) and Hct (1.3%) than that of the control group. An increase in biochemical indices of the non-anemic treatment indicates that this group's initial iron status was only marginally adequate.

Introduction

Iron deficiency anemia is widely prevalent around the world affecting about 700 to 800 million people in less developed countries and 60 to 70 million in developed countries[1,2]. On a regional basis, South Asia and Africa have the highest prevalence with an estimated rate of more than 40% in all age groups except for adult males and pregnant women, the latter group is the most vulnerable to anemia with an estimated prevalence rate of more than 65% in South Asia[1-3].

In Pakistan, iron deficiency anemia is a major nutritional problem among all age groups. The most vulnerable groups are preschool children and pregnant and lactating women. According to a recent National Nutritional Anemia Survey[4], iron deficiency in children has accounted for 83% of all anemia. Its main cause appears to be nutritional, ie dietary intake is insufficient to meet the physiological needs of the body. Other contributing factors considered to be responsible for iron deficiency anemia are low purchasing power of the people, low bioavailability of iron from cereal-based diets, poor dietary practices and poor hygiene and sanitation which increase the risk of infection and worm infestation.

There is increasing evidence[5-9] that iron deficiency not only impairs the immune function, cognitive and scholastic performance but also adversely affects the behavioral and physical activity of the children. In the North West Frontier Province, there is a paucity of data on the prevalence of iron deficiency anemia in different age groups and no study related to iron supplementation has been carried out on pre-adolescent girls. The present study is therefore designed to assess the prevalence of iron deficiency anemia in eight to 11 year old schoolgirls as well as to investigate the impact of iron supplementation on the biochemical indices of the iron status in these girls.

Materials and methods

Iron supplementation study was carried out in the Bannu district which is about 120 miles south of the North West Frontier Provincial capital, Peshawar. Permission to conduct a study in schools was sought from the District Education Officer of Bannu. Two girls' schools were chosen from which 220 eight to 11 year old girls were selected randomly after obtaining informed consent from their parents. All children were physically and clinically examined by the paediatrician and those having the symptoms of illnesses and nutritional deficiencies other than iron deficiency were excluded from the study.

One hundred and ninety-nine healthy children were left after initial screening. About 7 ml venous blood sample was obtained from each girl and put in serum separation and EDTA containing tubes for biochemical assays. Serum ferritin, hemoglobin, hematocrit, transferrin saturation, mean corpuscular volume, mean hemoglobin concentration and mean corpuscular hemoglobin concentration were determined by the

Correspondence address: Dr Parvez Iqbal Paracha, PO Box 981, Peshawar University, Peshawar, Pakistan.

Table 1. Baseline biochemical values* of the anemic and non-anemic groups.

Group	SF (ng/ml)	Hb (g/dl)	Hct (%)	TS (%)	MCV (fl)	MCH (pg)	MCHC %)
Anemic (n=69)	9.54^{a}†±2.08	11.16^{a}±1.15	34.33^{a}±1.24	12.83^{a}±1.35	76.61^{a}±1.18	24.90^{a}±1.72	32.48^{a}±1.18
Non-Anemic (n=70)	27.91^{b}±2.36	13.13^{b}±1.23	38.27^{b}±1.35	17.76^{b}±1.73	82.54^{b}±1.45	28.34^{b}±1.69	34.37^{b}±1.35
Anemic Treatment (n=35)	9.40^{a}±2.16	11.07^{a}±1.09	34.17^{a}±1.28	12.90^{a}±1.58	76.63^{a}±1.23	24.77^{a}±1.35	32.31^{a}±1.12
Anemic Placebo (n=34)	9.68^{a}±2.33	11.26^{a}±1.28	34.50^{a}±1.16	12.76^{a}±1.36	76.59^{a}±1.08	25.03^{a}±1.73	32.65^{a}±51.26
Non-Anemic Treatment (n=35)	26.66^{b}±2.31	13.07^{b}±1.25	38.20^{b}±1.29	17.59^{b}±1.85	82.11^{b}±1.70	28.23^{b}±1.47	34.25^{b}±1.16
Non-Anemic Placebo (n=35)	29.17^{b}±2.45	13.17^{b}±1.18	38.34^{b}±1.42	17.96^{b}±1.5	82.97^{b}±1.58	28.46^{b}±1.34	34.49^{b}±1.32

*Mean ± Standard Error of Mean.
†Means in columns with the same letter are not significantly different at $P<0.05$ level.

Table 2(a). Mean changes in biochemical values of the anemic and non-anemic groups after iron supplementation.

Group	SF (ng/ml)	Prob>[T]	Hb (g/dl)	Prob>[T]	Hct (%)	Prob>[T]	TS (%)	Prob>[T]
Anemic (n=59)	10.69	0.0001	0.72	0.0001	1.29	0.0001	2.15	0.0001
Non-Anemic (n=60)	4.63	0.0001	0.33	0.0001	0.67	0.0001	0.96	0.0001
Anemic Treatment (n=30)	20.00	0.0001	1.50	0.0001	2.73	0.0001	4.35	0.0001
Anemic Placebo (n=29)	1.07	0.1464	–0.08	0.5257	–0.21	0.5065	–0.13	0.5747
Non-Anemic Treatment (n=31)	8.58	0.0001	0.75	0.0001	1.55	0.0001	2.09	0.0001
Non-Anemic Placebo (n=29)	0.41	0.6919	–0.11	0.3120	–0.28	0.3921	–0.26	0.3559

Table 2(b). Mean changes in biochemical values of the anemic and non-anemic groups after iron supplementation.

Group	MCV (fl)	Prob>[T]	MCH (pg)	Prob>[T]	MCHC (%)	Prob>[T]
Anemic (n=59)	1.64	0.0001	1.17	0.0001	0.85	0.0001
Non-Anemic (n=60)	0.65	0.0001	0.43	0.0001	0.22	0.0001
Anemic Treatment (n=30)	3.70	0.0001	2.53	0.0001	1.77	0.0001
Anemic Placebo (n=29)	–0.48	0.3156	–0.24	0.3629	–0.10	0.5578
Non-Anemic Treatment (n=31)	2.00	0.0001	1.03	0.0001	0.54	0.0001
Non-Anemic Placebo (n=29)	–0.79	0.0800	–0.21	0.3259	–0.14	0.3548

methods as recommended by the International Committee for Standardization in Hematology[10-13].

For the purpose of categorizing the children into the anemic and non-anemic groups, iron deficiency anemia was defined as serum ferritin: ≤ 12 ng/ml and hemoglobin < 12 g/dl or hematocrit ≤ 35%. Of 199 children, 69 met the criteria of iron deficiency anemia and were randomly divided into treatment and placebo groups. The children whose biochemical values of iron status were above the standard cut off values were categorized as the non-anemic children. Of 88 non-anemic children, 70 children were randomly chosen and equally divided into treatment and control groups. Of the remaining 42 girls, 17 refused to give blood while 25 did not fall under the criteria of the anemic and non-anemic girls. The sensitivity of the diagnosing criteria was assessed by selecting a cut-off point of 1 g/dl rise in hemoglobin value of the anemic group following iron supplementation.

All children in the treatment and control groups received a multivitamin tablet, the treatment group however received an additional 76 mg of elemental iron in the form of ferrous gluconate tablets per day for 11 weeks. At the end of the supplementation period, about 7 ml of blood was collected from the treatment and control groups for biochemical assays. Twenty girls, 10 each from the anemic and non-anemic groups refused to give blood. Therefore, the final sample size was reduced from 139 children to 119 children. All the biochemical tests which were carried out before the iron supplementation were repeated in a similar fashion following the recommended procedures[10-13].

The biochemical data was entered into the computer for error checking and statistical analysis. A Statistical Analysis System (SAS) software program was used to perform the statistical analyses[14-15]. Analysis of variance was run on the baseline biochemical values to compare mean differences among the treatment and control groups. A paired-wise comparison of the mean difference between the biochemical values of the anemic and non-anemic groups was run to determine the effect of treatment on the biochemical parameters of the iron status. Analysis of co-variance was employed by keeping the pretreatment level of the iron parameter as a covariate for the corresponding post-treatment value and to compare the mean difference among the different groups.

Results

Table 1 depicts the baseline mean biochemical values of the anemic and non-anemic groups. As evident from the table, at the baseline there were significant ($P<0.05$) differences between the anemic and non-anemic groups in terms of their mean serum ferritin (SF), hemoglobin (Hb), hematocrit (Hct), transferrin saturation (TS), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) levels. However, there were no significant ($P>0.05$) differences in the mean biochemical values of the iron status between the treatment and placebo of the anemic and non-anemic groups.

Paired-wise comparisons (Table 2) within a group demonstrated that the administration of iron resulted in a significant increase in SF (20 ng/ml), Hb (1.5 g/dl), Hct (2.73%), TS (4.35%), MCV (3.70 fl), MCH (2.53 pg) and MCHC (1.77%) levels of the anemic children. Iron treatment in the non-anemic children also resulted in an increase in their biochemical levels, but the change in this group was signficantly ($P<0.05$) lower than that in the anemic treatment group. Conversely, with the exception of SF, all other biochemical values of the placebo group were decreased by the end of the supplementation trial.

Table 3 shows the adjusted changes (T2-T1) in biochemical values of the anemic and non-anemic as well as the treatment and placebo groups. Iron supplementation resulted in significantly greater changes in biochemical values of the anemic treatment group compared to their corresponding non-anemic treatment as well as placebo groups. There was also a significant ($P<0.05$) interaction between the two independent variables (treatment × iron status) for all of the dependent variables of iron status. The interaction showed that treatment had a significantly higher effect on the biochemical values of the anemic group compared with those of the non-

Table 3. Adjusted mean changes in biochemical values* of the anemic and non-anemic groups after iron supplementation.

Group	SF (ng/ml)	Hb (g/dl)	Hct (%)	TS (%)	MCV (fl)	MCH (pg)	MCHC (%)
Anemic (n=59)	9.76[a]†± 1.52	0.67[a]± 0.14	1.12[a]± 0.28	2.51[a]± 0.31	1.00[a]± 0.38	0.81[a]± 0.25	0.41[a]± 0.14
Non-Anemic (n=60)	5.26[b]± 1.50	0.36[b]± 0.14	0.78[b]± 0.28	0.52[b]± 0.30	1.20[b]± 0.38	0.75[b]± 0.25	0.62[b]± 0.13
Anemic Treatment (n=30)	19.23[a]± 1.69	1.47[a]± 0.74	2.59[a]± 0.33	4.72[a]± 0.35	3.15[a]± 0.45	2.19[a]± 0.30	1.27[a]± 0.18
Anemic Placebo (n=29)	0.29[b]± 1.71	−0.13[b]± 0.16	0.36[b]± 0.34	0.31[b]± 0.38	−1.15[b]± 0.49	−0.57[b]± 0.29	−0.45[b]± 0.16
Non-Anemic Treatment (n=31)	9.28[c]± 1.58	0.78[c]± 0.16	1.69[a]± 0.32	1.70[c]± 0.35	2.51[a]± 0.43	1.34[a]± 0.28	0.92[a]± 0.16
Non-Anemic Placebo (n=29)	1.25[b]± 1.78	−0.07[b]± 0.17	−0.13[b]± 0.34	−0.66[b]± 0.36	−0.11[b]± 0.49	0.15[b]± 0.30	0.32[c]± 0.17

*Least Square Mean ± Standard Error of Mean.

†Means in columns with the same letter are not significantly different at $P<0.05$ level.

anemic group. The increase in the anemic treatment group was about twofold greater than that of the non-anemic treatment group. Conversely, the biochemical values of the placebo groups remained significantly lower than those of the treatment groups.

Using the established criteria (SF $\leq$ 12 ng/ml and Hb $<$ 12 g/dl or Hct $\leq$ 35%) of iron deficiency anemia, the prevalence of iron deficiency anemia in the study population was found to be 35% while a sensitivity of diagnosing criteria (based on an increase in Hb level of 1 g/dl following iron supplementation) in the anemic treatment groups was found to be 88%.

Discussion

The biochemical data indicate that the children in the study population were not severely anemic; most of the children were mild to moderately anemic. The lower severity of anemia in these children might be attributed to: i) the children's prepubertal age where the demand for iron is relatively small and thus the risk for anemia is low; and ii) the study site, ie the study was limited to urban schools where children had come from relatively well-off families. These children also had access to better health care facilities, better hygiene and sanitary conditions and the availability of animal products compared to their rural counterparts. Thus, the prevalence of iron deficiency anemia which we found in the urban schoolgirls may be lower than that prevalent in the rural population.

Changes in the biochemical values of the anemic and non-anemic groups following iron supplementation showed that the main effect of treatment to cause these changes was not equal in both the groups. The anemic children treated with iron had shown a significantly higher response to the treatment than their non-anemic peers. The notion that supplementation was more effective in the anemic group could be explained in part to its increased iron absorption and its effective utilization in the body. Our results of supplementation studies are in line with the results of other larger studies conducted in Indonesia and Thailand[16,17].

The results of this study clearly indicate that iron treatment was effective in correcting iron deficiency anemia in the pre-adolescent schoolgirls. The iron indices response to iron treatment was reasonably good so that one could eliminate the possibility of other anemia associated simultaneously with iron deficiency anemia. An increase in hemoglobin level of 1 g/dl in 88% of the anemic treatment children further confirms that these children were correctly diagnosed. The results also support the earlier researchers[7] hypothesis of an increase in Hb of 1 g/dl as a yardstick of measuring supplementation effectiveness.

It is also noteworthy to mention that there were decreases in the mean hemoglobin, hematocrit and transferrin saturation values of the control group despite having the normal intake of folate, B12 and other vitamins. This also suggests that the children of the control group were free from folate and B12 deficiencies; otherwise, hemoglobin and hematocrit values would have increased at the end of the trial. However, a decrease in the iron indices of the control group might be attributed to a decrease in the dietary iron intake of children during the iron supplementation period. The change in the body nutrient status is usually accompanied by a change in the dietary intake during seasonal variations.

Conclusion

A significant positive change in the biochemical indicators of the iron status in both the anemic and non-anemic children after iron supplementation indicates that the dose and duration of iron supplementation were well balanced. Iron treatment in the anemic children not only corrected the iron deficiency deficits but also significantly increased the biochemical values which reflects the body's iron status. An increased response to iron treatment in the anemic children was expected because of increased iron absorption and utilization in these subjects, which in turn varifies that the iron deficient anemic children were correctly diagnosed.

A moderate positive response to iron treatment in the non-anemic group could be attributed to the fact that pretreatment biochemical values of this group were marginally adequate and had a capacity to replenish and improve its iron status. In contrast, hemoglobin, hematocrit and transferrin saturation values of the anemic and non-anemic children treated with placebo decreased, even though these children received the RDA of folate and B12 during the experimental period. This suggests that these children were free from folate and B12 deficiencies, otherwise one would have observed an increment in their hematological values.

This study demonstrates that an optimal dose of iron supplementation is the fastest, safest and low-cost therapy for children and other vulnerable population groups who have increased risk of iron deficiency anemia. Iron supplementation is the short term prophylactic and therapeutic treatment to prevent and control iron deficiency anemia in the target population. The strategy of repleting the body's iron stores through iron supplementation may be of particular value to the health planners of less developed countries where bioavailability of iron from dietary sources is limited.

References

1 WHO. Global estimates for health situations assessment and projections. WHO, Geneva, 1990.
2 Demaeyer EM. Preventing and controlling iron deficiency anemia through primary health care: A guide for health administrators and programme managers. WHO, Geneva, 1989.
3 International Nutritional Anemia Consultative Group (INACG). A report of the ninth meeting of the INACG, Geneva, 1985.
4 Khan MA, Jalil F. Nutritional Anemia: Classification and effect of therapeutic trial for proposed fortification program. Nutrition Cell, Planning and Development Division, Islamabad, Pakistan, 1985.
5 Chandra RK, Saraya AK. Impaired immunocompetence associated with iron deficiency. J Pediatr 1975; 86:899–902.
6 Deinard AS, List A, Lingren B, Hunt JV, Chjang PN. Cognitive deficits in iron deficient and iron deficient anemic children. J Pediatr 1986; 108:681–688.
7 Soemantri AG, Pollitt E, Kim I. Iron deficiency anemia and educational achievement. Am J Clin Nutr 1985; 42:1221–1228.

8 Pollitt E, Greenfield D, Leibel R. Behavioral effects of iron deficiency among preschool children in Cambridge, Mass. Fed Proc 1978; 37:A487.
9 McDonald R, Strause L, Hegenaure J, Saltman P, Sucec AA. Limitations to maximum exercise performance: Implications of iron deficiency. In Dotson CO and Humphrey JH eds Exercise Physiology. New York, 1985.
10 Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Lewellin P. An immonoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. J Clin Pathol 1972; 25:326–329.
11 International Committee for Standardization in Haematology. Preparation, characterization, and storage of human ferritin for use as a standard for the assay of serum ferritin. Clin Lab Haemol 1984; 6:177–191.
12 International Committee for Standardization in Haematology. Recommendations for hemoglobinometry in human blood. Br J Haematol 1967; 13:71–75.
13 International Committee for Standardization in Haematology. Recommendations for measurement of serum iron in human blood. J. Clin Pathol 1971; 24:334–335.
14 SAS User's Guide: Statistics, Version 5 Edition, SAS Institute, Inc. Box 8000, Cary, North Carolina, 1985; 2:7511-8000.
15 Snedecor GW, Cohran WG. Statistical Methods, 7th edition, The Iowa State University Press, 1980.
16 Soemantri AG. Preliminary findings on iron supplementation and learning achievement of rural Indonesian Children. Am J Clin Nutr (Suppl) 1989; 50:698–702.
17 Pollitt E, Hathirat P, Kotchabhakdi NJ. Missell L, Valyasevi A. Iron deficiency and educational achievement in Thailand. Am J Clin Nutr (Suppl) 1989; 50:687–697.

Effect of iron supplementation on biochemical indices of iron status in selected pre-adolescent schoolgirls in North West Frontier Provice, Pakistan

Parvez I. Paracha, S.M. Khan, I. Ahmad and G. Nawab

Asia Pacific Journal of Clinical Nutrition 1993; 2:177–181

補充鐵對巴基斯坦東西邊境省青春期前女學生鐵的生化指標的影響

摘要

作者研究了8-11歲女學生缺鐵性貧血 (IDA) 的發病率，並研究了補充鐵對鐵的生化指標的影響。凡血清鐵蛋白水平 (SF) 等於或少於 12ng/ml 和血紅蛋白 (Hb) 少於 12g/100ml 或血細胞比容等於或少於 35% 的兒童被確定爲缺鐵性貧血。作者進行雙盲試驗，從治療和對照組隨機選擇貧血和非貧血兒童，所有組每日服用多種維生素片，治療組每日另加元素鐵76 毫克共 11 週。這些兒童有 35% 患有缺鐵性貧血，貧血治療組經補充元素鐵後較對照組有明顯的改變，其 SF，Hb 和 HCt 分別增加20ng/ml，1.5g/100ml 和 3% ($p<0.05$)。非貧血治療組經補充元素鐵後較對照組亦有明顯的改變，其 SF， Hb和 HCt分別增加 9ng/ml，0.78g/100ml 和 1.3%，非貧血治療組生化指標的增加說明了該組兒童鐵營養狀況僅勉強足夠。