INVESTIGATION OF THE CAUSES OF SUBOPTIMAL HAEMOGLOBIN RESPONSE TO IRON SUPPLEMENTATION

PRICHA CHAROENLARP, SRI SRINOPHAKHUN*, PENSRI POOTRAKUL**, TIEN POLPOTHI and PRAMUALSRI CHATPUNYAPORN

Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, *Department of Parasitology, Faculty of Public Health, and **Thalassemia Center, Faculty of Postgraduate Studies, Mahidol University, Bangkok, Thailand.

INTRODUCTION

Iron deficiency anaemia is common the world over especially in developing countries (WHO, 1975). It has been estimated that more than 500 million people are iron deficient (Finch and Huebers, 1982). Intervention, however, is not a simple matter. In WHO−supported supplementation trials in Mauritius (Scott, 1960), Israel (Izak *et al.*, 1973), India (Sood *et al.*, 1975), Burma and Thailand (Charoenlarp *et al.*, 1988), a number of subjects were found to be anaemic. In the case of Thailand more than 20% of men and non-pregnant and pregnant women with normal haemoglobin typing remained anaemic after supplementation with a daily dose of 120−240 mg of iron with folic acid for a period of 12−15 weeks. It was hypothesized that this was due to environmental factors, particularly nutritional status and the presence of infection. It was decided to investigate the effect of providing protein, vitamins and minerals in addition to iron supplementation on the haemoglobin concentration of children with normal haemoglobin typing.

This investigation received financial support from Nestle Nutrition, Switzerland.

MATERIALS AND METHODS

Subjects : Six primary schools were selected from Bangkok Metropolitan area and two rural districts of Nakhon Pathom Province, 70 kilometers west of Bangkok.

Schools 1 and 2 are located in urban central Bangkok, school 3 and 4 in rural areas of Huay Kwang District, schools 5 and 6 in rural areas of Don Khoy District. The selection of rural control and supplemented groups from each rural district was based on the ease of administration of the protein supplement.

With the consent of their parents, all the children were screened to determine health status and haemoglobin typing. Only those in good health were included; those with thalassaemia disease, abnormal haemoglobinopathies or beta-thalassaemia trait were excluded. The study was approved by the ethical committee and district authorities on health and education.

Blood examination : Venous blood was collected in a lying position between 9−11 a.m. after 15 minutes' rest. Blood examinations were carried out on three occasions; before supplementation and after the first and second supplementation phases. Subjects with a history of blood loss or surgery were excluded from the study. Blood samples were taken only from those who had no history of illness during the previous three weeks and who appeared healthy at the time of blood collection.

Haemoglobin typing was carried out by cellulose acetate electrophoresis and the quantity of HbA_2 was estimated by eluting the cut-out strips (Dacies and Lewis, 1984). Detection of alpha-thalassaemia−1 trait was carried out by the brilliant cresyl blue staining technique only in those who remained anaemic after the second phase.

Haemoglobin was estimated in duplicate by the standard cyanmethaemoglobin method using a Perkin-Elmer Model 35 Digital Spectrophotometer, and checked against WHO reference standards. Haemoglobin concentration was determined after each phase of supplementation.

Stool examination : Stools were examined for hookworm eggs by Kato's thick-smear technique (Martin and Beaver, 1968). After first stool examination, all children received a single 400 mg dose of albendazole. The children whose first stools were positive for hookworm eggs had a follow-up stool examination, and those who were still positive received a second dose of albendazole.

Albendazole was given to all the children in the rural areas before the start of the second phase of supplementation.

Supplementation : Iron tablet: each contained: dried ferrous sulphate BP 200 mg, and folic acid BP 80 0.250 mg. These tablets were manufactured by Weiders Farmasoytiske A/S, Oslo, Norway.

Vitamin and mineral tablest: two tablets containing:

Vitamin A	2500 IU
Vitamin D	5 µg
Vitamin E	20 mg
Vitamin C	50 mg
Vitamin B_1	2 mg
Vitamin B_2	5 mg
Niacin	11 mg
Vitamin B_6	2 mg
Calcium pantothenate	4 mg
Vitamin B_{12}	5 µg
Biotin	100 mg
Zinc	2.5 mg
Manganese	0.5 mg
Copper	0.5 mg
Molybdenum	0.15 mg

These tablets were manufactured by the Hoffmann-La Roche Company, Basle.

Soybean milk: proximate analysis (% w/w) protein 2 g%, fat 1 g%, and carbohydrate 6 g%.

The children, aged 6–11 years, were divided into 3 groups, each consisted of about 200 children from the primary schools and the supplement regimen given was as follows:

Group	First phase (60 days)	Second phase (60 days)
1. Urban control (moderate socioeconomic status)	Two tab. of 60 mg iron with 0.25 mg folic acid per day.	Two tab. of 60 mg with 0.25 mg folic acid per day.
2. Rural control (low socioeconomic status)	Two tab. of 60 mg iron with 0.25 mg folic acid per day.	Two tab. of 60 mg iron with 0.25 mg folic acid per day.
3. Rural study (low socioeconomic status)	Two tab. of 60 mg iron with 0.25 mg folic acid per day.	Two tab. of 60 mg iron with 0.25 mg folic acid per day plus protein, vitamin and mineral supplements.

During the first phase, each child received one iron tablet at 8:00 a.m. and another at 1:00 p.m. before entering class of school days (five days a week) for a period of 60 days. The tablets were swallowed in front of a supervisor.

In the second phase, each child in the supplemented group was given 100 g of meat (3 days a week) or one egg (two days a week), 200 ml of soybean milk, and two vitamin and mineral tablets at 4:00 p.m. (after the afternoon class).

Statistical analysis : Means and standard deviations were calculated for the hemoglobin concentration of the children in each school and analysis of variance was used to compare the mean haemoglobin concentrations.

RESULTS

Table 1 shows the results of the haematological determinations and the stool examinations of the children in each primary school at the onset of the study. The mean haemoglobin concentrations ranged between 12.3–12.8 g/dl. Anaemia prevalences (haemoglobin less than 12 g/dl) ranged between 13–31%; 82.9% of the children had normal haemoglobin typing (AA) and 14.8% were HbE heterozygotes (AE). The hookworm infection rate in the rural groups was 56% but the infection load was mild (fewer than 1,000 eggs per gram of faeces).

Only the children with normal haemoglobin typing were included in the study. Among the drop-outs, one child could not tolerate the iron tablets and six moved to other areas during the trial. Still others had undergone only one follow-up blood examination due to their absence, sickness or convalescence at the time of blood collection. None of the children, including the drop-outs, were anaemic. Only those children who had undergone three blood examinations were included in the analysis of the results, shown in Table 2. Their height and weight are shown in Table 3.

After the first phase of supplementation,

Table 1

Haematological findings and results of stool examinations of all the children studied.

	Urban (Bangkok)		Rural (Nakhon Pathom) Area A		Area B	
School	1	2	3	4	5	6
Number	111	110	132	106	118	120
Haemoglobin						
$\bar{X} \pm$ SD g/dl	12.55 ± 0.81	12.47 ± 0.94	12.37 ± 1.02	12.28 ± 0.90	12.78 ± 0.75	12.36 ± 0.90
Anaemia prevalence						
Hb below 12 g/dl	20.7%	26.6%	25.0%	31.1%	12.8%	30.3%
Hb typing						
AA	93	88	104	81	104	107
AE	16 (14.4%)	18 (16.4%)	24 (18.2%)	22 (20.8%)	11 (9.3%)	12 (10.0%)
EE	1	1	2	–	–	–
Other abnormal Hb	–	1	–	–	1	–
Beta-thal trait	1	1	1	2	2	1
Thalassaemia	–	1	1	1	–	–
Hookworm infection	0%	1%	64%	44%	48%	64%

there was significant increase in mean haemoglobin concentrations of the children in all schools; there was a statistically significant difference in mean haemoglobin concentrations between the groups ($p < .05$ by analysis of variance). Overall anaemia prevalence decreased from 19.0% to 4.9%.

After the second phase, there was significant difference in mean haemoglobin concentrations between schools ($p < .05$ by analysis of variance). The mean haemoglobin concentration of the children in urban control was higher than those of the rural groups. Only 1.5% of all children had haemoglobin concentrations below 12 g/dl and half displayed alpha-thalassaemia-1 traits (Table 4).

DISCUSSION

In selecting children with normal haemoglobin typing, those with alpha-thalassaemia traits were not excluded because the method for detecting alpha-thalassaemia-1 trait is time-consuming and a practical method for detecting alpha-thalassaemia-2 trait is not available. HbE is common in Thailand, the prevalence being about 13.5% (Wasi *et al.*, 1969). In this study, the mean haemoglobin concentrations of 103 children with HbE and eight children with beta-thalassaemia traits were respectively 0.7 and 1.7 g/dl lower than that of children with normal haemoglobin type; 48.5% of children with HbE and seven out of eight children with beta-thalassaemia traits were anaemic.

The high prevalence of hookworm infection in areas of Thailand–40% using Kato's thick smear technique (Preuksaraj *et al.*, 1982) was aslo observed among the children

Table 2

Haematological findings of the children** before supplementation (I), after first phase (II)*** and after second phase (III).****

Group	Urban control (School 1 and 2)	Rural control (School 3 and 5)	Rural study (School 4 and 6)
Number	165	200	182
Haemoglobin I	12.69 ± 0.06*	12.71 ± 0.06	12.45 ± 0.06
Haemoglobin II	13.27 ± 0.06	13.39 ± 0.06	13.23 ± 0.06
Haemoglobin III	13.78 ± 0.06	13.61 ± 0.05	13.59 ± 0.06
Prevalence of anaemia I	16.4%	13.5%	27.4%
Prevalence of anaemia II	4.8%	4.5%	5.5%
Prevalence of anaemia III	1.2%	1.0%	2.2%

* Expressed as mean ± SEM g/dl.

** Only those children who had three blood examinations.

*** Iron supplementation with folate for all.

**** Iron supplementation with folate for all, and protein, vitamins and minerals for those in the supplemented rural group.

Table 3

Height and weight of the children* before supplementation (I), after first phase (II) and after second phase (III).

Group	Number	Age** (median yr)	Height (mean ± SD cm) I	II	III	Weight (mean ± SD kg) I	II	III
Urban control	131	8.7	128.00 ±8.19	129.54 ±8.02	131.2 ±8.43	25.44 ±5.55	26.47 ±5.58	27.71 ±6.46
Rural control	178	8.9	125.78 ±6.58	126.69 ±6.67	128.19 ±6.94	22.86 ±3.16	24.02 ±3.45	24.45 ±3.60
Rural study	170	8.7	124.05 ±8.02	125.95 ±7.92	127.13 ±8.37	23.08 ±4.27	23.99 ±4.45	24.66 ±4.84

* Only those who were measured.

** At the time of the first measurement.

Table 4

Detection of inclusion body for alpha-thalassaemia-1 traits in the children who had haemoglobin below 12 g/dl after supplementation.

Case No.	Group	Final haemoglobin g/dl	Inclusion body
1	urban control	11.9	Negative
2	urban control	11.7	Positive
3	rural control	11.3	Positive
4	rural control	11.5	Positive
5	rural study	11.8	Not done
6	rural study	11.4	Positive
7	rural study	11.6	Negative
8	rural study	11.7	Negative

studied; all were treated with anthelmintics every 4 months.

The mean haemoglobin concentrations of the children in each school after the first phase suggest that there were probably different haemoglobin responses after short term supplementation. However, a further increase in mean haemoglobin concentrations in the second phase cancelled out the difference in mean haemoglobin between the rural control and the rural study groups. The higher mean haemoglobin concentration of the urban control group is probably due to their higher socioeconomic status which was shown by their higher height and weight (Table 3).

Hallberg *et al.*, (1968) found that 2% of healthy Swedish women with normal iron stores had haemoglobin concentrations below 12 g/dl, which corresponds to the results of Garby *et al.*, (1969) after the iron supplementation of Swedish women. In the present study, only 1.5% of healthy children had a haemoglobin concentration below 12 g/dl after iron supplementation.

The incidence of folate deficiency anaemia is quite low in Thailand. Folate supplementation studies have shown that there is no effect on haemoglobin concentration in non-pregnant and pregnant women (Charoenlarp *et al.*, 1988) and in children with thalassaemia (Vorawan Tanphaichitr, pers. commun.). The effects of iron tablets with folate in this study are likely due to elemental iron only.

SUMMARY

The investigation concerned 572 children, 6–11 years of age, who were divided into three groups: urban control, rural control and rural study. During the first phase of supplementation, each child received 120 mg of elemental iron (as ferrous sulphate) with 0.50 mg of folic acid daily for 60 school days (five days a week) which resulted in a suboptimal haemoglobin concentration. During the second phase, all groups continued to take iron-folate tablets for 60 school days while the children in the study group also received protein, vitamins and minerals. Final blood examinations showed no significant difference in mean haemoglobin concentrations between the rural study

and rural control groups. Only 1.5% of all children had haemoglobin below 12 g/dl. It can therefore be concluded that strict supervision of iron tablet intake and long-term supplementation with iron are essential for optimal haemoglobin response.

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