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ORIGINAL ARTICLE

Impact of weekly iron folic acid supplementation with and without vitamin B12 on anaemic adolescent girls: a randomised clinical trial

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BACKGROUND/OBJECTIVES: In India, approx. 70% of the adolescent girls are anaemic (haemolgobin < 120 g/l). The present study was a supervised randomised double-blind clinical trial conducted among adolescent girls (11–18 years) to assess and compare the impact of weekly iron folic acid (IFA) supplementation with or without vitamin B12 on reduction in the prevalence of anaemia and on blood/serum levels of haemoglobin, serum ferritin, folic acid and vitamin B12.

SUBJECTS/METHODS: Community-based randomized controlled trial was carried out in Kirti Nagar slums of West Delhi. A total of 446 mild (100–119 g/l) and moderate (70–99 g/l) anaemic volunteer adolescent girls were identified and randomised into two groups. Weekly supervised supplementation was given for 26 weeks: Group A ($n = 222$): iron (100 mg), folic acid (500 mcg) and placebo; Group B ($n = 224$): iron (100 mg), folic acid (500 mcg) and cyanocobalamin (500 mcg for 6 weeks and 15 mcg for 20 weeks). Haemoglobin, serum ferritin, folic acid and vitamin B12 levels were assessed at baseline and after intervention. A total of 373 subjects completed 26 weeks of supplementation successfully.

RESULTS: The mean haemoglobin increased from 106.7 ± 11.2 g/l and 108.9 ± 8.91 g/l in Group A and Group B at baseline to 116.4 ± 10.8 g/l ($P < 0.001$) and 116.5 ± 10.26 g/l ($P < 0.001$) at post-intervention, respectively, with the reduction in the prevalence of anaemia by 35.9% in Group A and 39.7% in Group B ($P > 0.05$). A total of 63.3% participants had deficient vitamin B12 levels (< 203 pg/ml) at baseline, which reduced to 40.4% after intervention with cyanocobalamin, whereas no change was observed in vitamin B12 status in the other group. Significant reduction ($P = 0.01$) in the prevalence of serum ferritin deficiency (< 15 ng/ml) was observed in the group supplemented with vitamin B12 (from 36.5 to 6.4%) as compared with the other group supplemented with only IFA (from 39.1 to 15.2%).

CONCLUSIONS: IFA supplementation with or without vitamin B12 is an effective measure to cure anaemia. Although addition of vitamin B12 had similar impact on improving haemoglobin status as IFA alone, it resulted in better ferritin status. Hence, more multi-centre studies with a longer duration of supplementation or higher dose of vitamin B12 may be undertaken to assess the possible impact of vitamin B12 on improving haemoglobin levels in the population.

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INTRODUCTION

Iron deficiency anaemia is a major public health problem in developing countries. The consequences of anaemia include impaired cognitive performance, significant reduction in physical work capacity and productivity, increased morbidity from infectious diseases, greater risk of death among the pregnant women during the perinatal period, intrauterine growth retardation, low birth weight and prematurity.[1]

Adolescent girls constitute an important physiological group, and their nutritional requirements demand special attention as they are future mothers.[2] The median age of marriage in India is around 18 years. When a woman enters pregnancy with a large iron deficit and is subjected to the added demands for iron during pregnancy, it may be too late to address the problem of anaemia.[2]

In India, available literature has indicated the prevalence of anaemia among adolescent girls to vary between 56 and 98%.[3–6] In the last decade, many efforts have been made to address the problem of anaemia among adolescent girls. The National Nutritional Anaemia Control Programme (now known as National Iron Plus Initiative) of Ministry of Health and Family Welfare, Government of India, included adolescent girls as a beneficiary in 2007.[7] The recommended frequency of dosage is weekly supplementation of 100 mg of iron and 500 mcg of folic acid[8] besides biannual deworming. Despite the efforts made by the Government of India to reduce the prevalence of anaemia, it still continues to be very high.

It is well documented that the maintenance of the normal haematopoietic function also requires adequate levels of many other nutrients like folate and vitamin B12.[9] Vitamin B12 (cobalamin) and folate are essential in several metabolic pathways in the central nervous system, and their metabolism is intimately connected.[10] Both are involved in single-carbon transfer (methylation) reactions necessary for the production of monoamine neurotransmitters, phospholipids and nucleotides.[11]

Studies have shown that vitamin B12 status has an impact on the folic acid metabolism in the body. Orally administered or

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injected pteroylglutamic acid (folic acid) has been reported to disappear rapidly into the tissues of vitamin B12-deficient patients, as manifested by rapid disappearance of *Streptococcus faecalis* activity from serum and urine.[12,13] Hence, vitamin B12 deficiency can have profound impact on folate metabolism.[14] There is little information on whether vitamin B12 supplements improve the effects of iron supplements on haemoglobin synthesis and on anaemia.[15]

The present study was a *supervised* randomised double-blind clinical trial conducted to assess and compare the effectiveness of weekly iron folic acid (IFA) supplementation with or without vitamin B12 in reducing the prevalence of anaemia and on improving status of other haematological parameters like serum ferritin, folic acid and vitamin B12 among adolescent girls. The study also made an attempt to examine the possible role of vitamin B12 supplementation (cyanocobalamin) on haematological response among mild and moderately anaemic adolescent girls.

MATERIALS AND METHODS

The study was carried out in Jhuggi Jhompri (JJ) cluster (urban slum) at Kirti Nagar, New Delhi, which is a notified JJ colony as per *'Delhi Urban Shelter Improvement Board'* under Government of NCT of Delhi consisting of approximately 11 500 small units with a total population of approx. 60 000. The slum is densely populated having a poor hygiene, sanitation, ventilation and drainage facilities. Kirti Nagar JJ cluster is divided into 10 camps/sub-areas. Door-to-door survey to identify adolescent girls was carried out in 6 out of the 10 major blocks of Kirti Nagar slums.

The sample size was calculated with an assumption of reducing the prevalence of anaemia by 30% with 95% level of confidence and 90% power of test. The estimated sample size was 144 adolescent girls in each arm of the study. Assuming loss to follow-up mainly due to migratory nature of the slum dwellers, approx. 225 mild and moderately anaemic unmarried adolescent girls who volunteered were enrolled in each of the two groups.

By door-to-door survey in 6 blocks of Kirti Nagar, a total of 1228 adolescent girls were contacted and explained the purpose of the study, out of which 794 volunteered for the study, and their parents gave written consent. Haemoglobin levels were assessed for all 794 participants, and those found mild (70–99 g/l) or moderately (100–119 g/l) anaemic ($n = 466$) were scrutinised for inclusion criteria of being apparently healthy, unmarried and non-pregnant. Finally, a total of 446 (Group A: 222; Group B: 224) adolescent girls were randomly allocated into two groups (Figure 1). After 26 weeks of intervention, the final blood sample could be obtained from 373 participants (Group A: 184; Group B: 189). The data collection was initiated in January 2012 and completed in March 2013.

Regulatory approvals

Before the initiation of the study, Ethics Committee Clearance was obtained from Lady Irwin College, University of Delhi. Permissions were also obtained from Drug Controller General of India (DCGI) for conducting the trial on human volunteers and from State Licensing Authority, Bhopal, Madhya Pradesh, for manufacturing of cyanocobalamin (500 and 15 mcg) capsules. The trial was registered at Clinical Trial Registry of India under Indian Council of Medical Research, Ministry of Health and Family Welfare, Government of India, New Delhi (ID: CTRI/2011/12/002217), and at Clinical Registry at National Institute of Health (ID: NCT01490944). Data Safety Monitoring Board (DSMB) was constituted who met as per requirement

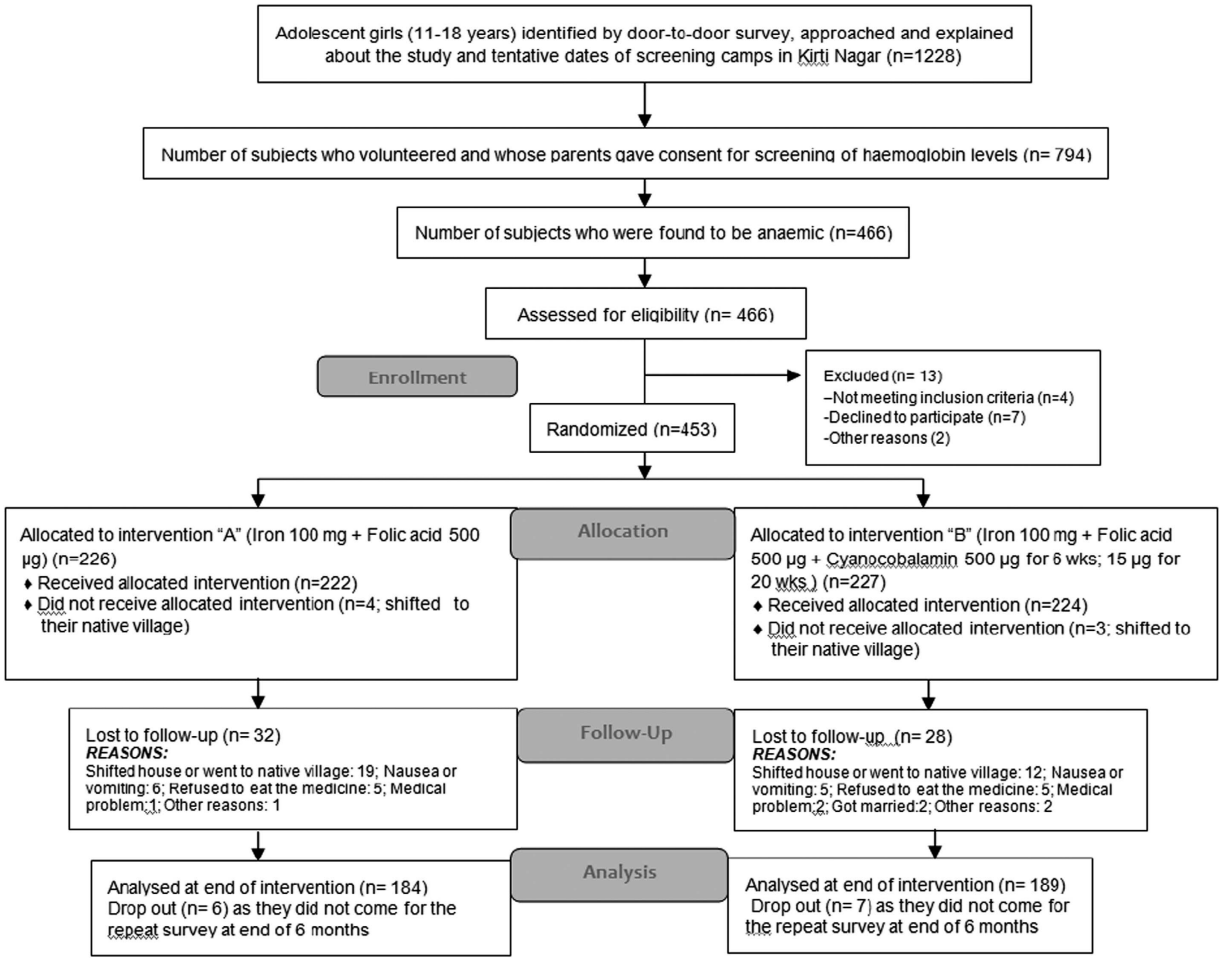

Figure 1. CONSORT flow diagram depicting overall recruitment, retention and follow-up status of the participants.

during the course of the study to review the progress and to study the drop-out status.

Parent's consent and subject's assent in the presence of a witness (person from the community) were dully taken before enrolling them for the study and at the time of repeat survey. Information sheet was also given to the participants bearing the contact details of the person to be contacted in case of any medical emergency.

Double blinding was carried out by a person having no conflict of interest in the study. The codes were sealed and kept with the person responsible for blinding as well as with the Chairperson of the DSMB. Decoding was performed after the repeat survey when all biochemical analysis was over and results were submitted to the Board.

Methodology

Mild (100–119 g/l) and moderately (70–99 g/l) anaemic adolescent girls (11–18 years) were identified on the basis of their haemoglobin levels. Those found to be non-anaemic were advised to get their haemoglobin regularly checked every 3 months and were counselled about the rich sources of iron in the diet. Severely anaemic girls were immediately referred to the nearest health centre for treatment. Mild and moderately anaemic adolescent girls were then randomly allocated to two study groups using computer-generated list by the person responsible for double blinding. One of the groups was supplemented with 100 mg of ferrous sulphate, 500 mcg of folic acid and placebo (lactose IP). The other group was supplemented with the same dose of IFA along with cyanocobalamin. For the first 6 weeks, 500 mcg cyanocobalamin and for the remaining 20 weeks 15 mcg cyanocobalamin was supplemented. The supplementation was given weekly for 26 weeks. As the study was double-blind, placebo and cyanocobalamin capsules were of the same colour, size, shape and odour. The tablets/capsules (that is, IFA tablets; cyanocobalamin 500 mcg; cyanocobalamin 15 mcg and placebo) were supplied by M/s Cyano Pharma Pvt. Ltd, Indore, Madhya Pradesh. Deworming tablet *'Zybend'* containing 400 mg Albendazole manufactured by M/s Zydus Cadila Healthcare Limited was given to all the participants in both the groups before initiation of IFA supplementation.

Intervention

The intervention was completely supervised. The supplementation was given once weekly by following door-to-door approach. The supplements and drinking water were given to the participants in the presence of the investigator. Incase the study volunteer was not available on the main day of the supplementation, a spare day was kept during the week. To ensure 100% compliance, a tentative time table of the study volunteer's activities during evening was also maintained, and they were followed in their *'Tuition classes', 'Salai Centres'*, etc for the supplementation.

Laboratory investigations

The biochemical estimation was carried out at the initiation of the study and after the intervention of 26 weeks. The analytes estimated include haemoglobin, serum ferritin, serum folic acid, serum vitamin B12 and C-reactive protein (CRP). Venous blood (5 ml) was drawn by trained personnel and collected in both EDTA and plain vacutainers (Beckton Dickinson, Franklin Lakes, NJ, USA) for the biochemical investigations. Blood in EDTA vial was kept for haemoglobin estimation. From blood collected in plain vial, serum was separated and stored in pre-labelled eppendorf vials. The serum and whole blood were stored in dry ice in a thermocol box and transported to the laboratory situated at a distance of 5 km from the field, where the samples were stored at − 80 °C till analysis.

The biochemical analysis was carried out at 'Centre for Promotion of Nutrition Research and Training, with special focus on North-East, Tribal and Inaccessible Population' (Indian Council of Medical Research), New Delhi. The laboratory of the Centre is accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) under ISO 15189:2007. Strict internal and external quality control measures were followed. Internal quality control was maintained using standards/controls with every batch of samples, whereas for external quality control the laboratory had enrolled in External Quality Assurance Programmes offered by AIIMS, New Delhi (for haemoglobin), and Bio-Rad India Pvt. Ltd (for serum ferritin, folate and vitamin B12).

Haemoglobin was estimated using direct cyanmethemoglobin method.[16] Serum ferritin, folic acid and vitamin B12 were estimated using chemiluminescence-based analyser (Immulite-1000, Siemens, Berlin, Germany). The analysis is a solid-phase, two-site chemiluminescent immunometric method. Serum ferritin is an acute phase reactant protein, whose value increases during infection; hence, CRP was also analysed in a sub-sample of 268 randomly selected participants (Group A: 138; Group B: 130) at post-intervention.

Statistical analysis

Data were entered using Microsoft Office Excel 2007 and were then exported to SPSS Statistics 20 (IBM, New Delhi, India) for analysis. A paired *t*-test was used to compare the haemoglobin, serum ferritin, folic acid and vitamin B12 levels before and after the intervention within the groups. Independent *t*-test was used to compare the biochemical levels between the groups. Chi-Square was used to compare the prevalence before and after intervention. The distribution for serum ferritin and vitamin B12 was found to be skewed; and hence, the values for these biomarkers were log transformed.

RESULTS

A total of 794 girls were screened for anaemia by haemoglobin estimation, out of which 466 were found to be anaemic (58.7%). WHO[17] classification was used to assess the severity of anaemia. Prevalence of mild, moderate and severe anaemia was reported as 46.5, 11.9 and 0.25%, respectively. Table 1 shows the groupwise distribution of the participants, their mean anthropometric measurements and biochemical parameters at baseline, indicating no significant differences between groups in major socio-demographic or nutritional characteristics, except that Group A participants were taller.

On classifying the participants on the basis of age (Table 2), it was observed that the prevalence of anaemia increased with age from 11 years (55.5%) to 16 years (63.9%). The mean haemoglobin levels also showed a progressive decline in this age group. Out of 794 participants screened, 419 (52.8%) had attained menarche. The mean haemoglobin level among those who had attained menarche (114.8 ± 14.99 g/l) was found to be significantly lower

Table 1. Biochemical parameters and anthropometric measurements at baseline

	Group A (IFA+Placebo) (n = *222*) *Mean ± s.d. (95% CI)*	*Group B (IFA+Cyanocobalamin)* (n = *224*) *Mean ± s.d. (95% CI)*	P-*value*
Mean age (years)	13.5 ± 1.94 (13.2–13.7)	13.6 ± 2.06 (13.4–13.9)	0.52
Mean weight (kg)	36.5 ± 7.22 (35.5–37.5)	35.8 ± 7.61 (34.8–36.8)	0.29
Mean height (cm)	143.8 ± 6.28 (142.9–144.6)	142.5 ± 6.49 (141.6–143.3)	0.04
Mean BMI (kg/m^2)	17.6 ± 2.89 (17.2–18.0)	17.5 ± 2.96 (17.1–17.9)	0.73
Mean haemoglobin (g/l)	106.6 ± 11.10 (105.1–108.1)	107.8 ± 8.97 (106.6–109.0)	0.22
Mean serum ferritin (ng/ml)	16.4 ± 2.80 (16.0–16.8)	18.2 ± 2.34 (17.9–18.5)	0.14
Mean serum folic acid (ng/ml)	7.9 ± 3.21 (7.5–8.3)	7.9 ± 3.35 (7.5–8.4)	0.97
Mean vitamin B12 (pg/ml)	151.4 ± 1.90 (151.2–151.7)	159.2 ± 1.88 (158.9–159.5)	0.39

Abbreviations: BMI, body mass index; CI, confidence interval; IFA, iron folic acid.

($P = 0.004$) than those who had not yet attained menarche (117.6 ± 11.71 g/l). Further, the prevalence of moderate anaemia among those who had attained menarche was 15.9%, whereas it was 7.5% in the other group, and this difference was found to be highly significant ($P < 0.001$).

Out of 26 scheduled doses, the average number of tablets consumed in Group A and Group B was 24.6 ± 2.6 and 24.8 ± 2.5 tablets, respectively. Out of 446 participants who enrolled, 373 participants (Group A: 184; Group B: 189) completed 26 weeks of supervised supplementation and were available at the time of repeat survey conducted after the completion of the intervention period. Hence, in all subsequent analysis, data for 373 participants are being compared. Figure 1 depicts the overall recruitment, retention and follow-up status of the participants.

Reduction in the prevalence of anaemia

Table 3 indicates the prevalence of anaemia in the two groups at baseline and post-intervention. A total of 37.9% (35.9% in Group A and 39.7% in Group B) participants who were anaemic (< 120 g/l) at baseline became non-anaemic (⩾120 g/l) after weekly supplementation for 26 weeks. The change in the prevalence of anaemia between the two groups was found to be similar ($P = 0.425$). Approx. 70% participants in both the groups showed an increase in haemoglobin values at post-intervention in comparison with their respective haemoglobin levels at baseline.

On assessing the migration of individual participant in terms of anaemia status and severity from their respective status at baseline, it was observed that, in the IFA and vitamin B12 group (Group B), out of 84.1% mild anaemic participants at baseline, 34.9% attained normal haemoglobin status (⩾120 g/l), 46.0% remained mild anaemic, whereas 3% shifted to moderate anaemic category at post-intervention. However, out of 15.9% volunteers who had moderate anaemia at baseline in IFA and vitamin B12 group, 4.7% were found to be non-anaemic, 8.4% shifted to mild category and 2.6% remained in moderate category of anaemia after 26 weeks of supplementation.

Similarly in the IFA group (Group A), at baseline 76.1% participants had mild anaemia, and 23.9% had moderate anaemia. Among those with mild anaemia, 29.3, 44.0 and 2.7% became non-anaemic, remained in mild category and shifted to moderate category, respectively, at post-intervention. Out of 23.9% moderate anaemic participants, 6.5, 13.0 and 4.3% attained normal haemoglobin levels, shifted to mild category and remained in moderate category, respectively.

Mean haemoglobin levels before and after intervention

The mean haemoglobin levels increased significantly at post-intervention in both the groups (Group A (IFA): 106.7 ± 11.2 g/l to 116.4 ± 10.8 g/l; Group B (IFA and Vitamin B12): 108.9 ± 8.91 g/l to 116.5 ± 10.26 g/l). Average increase in the mean levels of

Table 2. Haemoglobin levels according to age and menstrual status

Parameters	*No. of subjects*	*Anaemia*, n *(%)*				*Mean ± s.d. (g/l)*
		Any	*Mild*	*Moderate*	*Severe*	
Age (years)						
11	173	96 (55.5)	89 (51.4)	7 (4.0)	0 (0.0)	118.7 ± 9.92
12	144	81 (56.2)	68 (47.2)	13 (9.0)	0 (0.0)	117.1 ± 12.40
13	120	72 (60.0)	57 (47.5)	14 (11.7)	1 (0.8)	115.9 ± 13.68
14	100	62 (62.0)	44 (44.0)	18 (18.0)	0 (0.0)	113.7 ± 14.99
15	90	58 (64.4)	39 (43.3)	18 (20.0)	1 (1.1)	113.0 ± 15.16
16	83	53 (63.9)	43 (51.8)	10 (12.0)	0 (0.0)	113.9 ± 14.20
17	59	29 (49.2)	19 (32.2)	10 (16.9)	0 (0.0)	118.8 ± 16.45
18	25	15 (60.0)	10 (40.0)	5 (20.0)	0 (0.0)	115.4 ± 17.14
Menarcheal status						
Attained menarche	419	254 (60.6)	186 (44.5)	67 (15.9)	1 (0.2)	114.8 ± 14.99[a]
Not attained menarche	375	212 (56.5)	183 (48.8)	28 (7.5)	1 (0.3)	117.6 ± 11.71[a]

$n = 446$; figure in parentheses indicate percentage. [a]Cutoffs used are as follows: anaemia (Hb: < 120 g/l); mild anaemia (Hb: 100–119 g/l); moderate anaemia (Hb: 70–99 g/l); severe anaemia (< 70 g/l) (Source: WHO, 2001).

Table 3. Prevalence of micronutrient deficiencies before and after intervention

	Cutoff	*Group A (IFA+Placebo)* (n = *184*) *Prevalence (%)*			*Group B (IFA+Cyanocobalamin)* (n = *189*) *Prevalence (%)*			P-*value*[a]
		Baseline (BL)	*Post-intervention (PI)*	*Change in prevalence (BL-PI)*	*Baseline (BL)*	*Post-intervention (PI)*	*Change in prevalence (BL-PI)*	
Any anaemia	< 120 g/l[42]	100.0	64.1	35.9	100.0	60.3	39.7	0.425
Mild anaemia	100–119 g/l	76.1	57.1	19.0	84.1	54.5	29.6	0.750
Moderate anaemia	70–99 g/l	23.9	7.1	16.8	15.9	5.8	10.1	0.101
S. ferritin	< 15 ng/ml[42]	39.1	15.2	23.9	36.5	6.4	30.1	0.010
S. folic acid	< 4 ng/ml[43]	5.5	0.0	5.5	4.9	0.0	4.9	0.512
S. Vit B12	< 203 pg/ml[43]	63.6	63.6	0.0	63.3	40.4	22.9	< 0.001

Abbreviation: IFA, iron folic acid. [a]*P*-values signify the comparison between the change in the prevalence in Group A vs Group B.

haemoglobin was found to be similar ($P = 0.098$), being 9.7 ± 13.7 g/l in Group A and 7.5 ± 11.4 g/l in Group B.

On assessing the impact of IFA with or without vitamin B12 supplementation on the basis of haemoglobin levels at baseline, it was observed that lower the haemoglobin level at baseline, the greater the increase at post-intervention (Table 4).

The analysis further revealed that, as the prevalence of anaemia declined and mean haemoglobin increased, the entire curve for haemoglobin shifted to the right (Figure 2), indicating that not only anaemia prevalence decreased but the level of severity also decreased.

Status of other haematological parameters, that is, serum ferritin, folic acid and vitamin B12

The prevalence of serum ferritin deficiency (< 15 ng/ml) reduced from 39.1 to 15.2% and from 36.5 to 6.4% at post-intervention in Group A (IFA) and Group B (IFA+vitamin B12), respectively (Table 3). It was found that more participants in Group B as compared with Group A had attained normal serum ferritin levels (⩾15 ng/ml) at post-intervention, and the difference was statistically significant ($P = 0.010$). None of the participants had deficient serum folic acid levels (< 4 ng/ml) at post-intervention in both the groups. At baseline, 63.6% participants in the IFA group had suboptimal vitamin B12 levels (< 203 pg/ml), which remained the same at post-intervention. However, in Group B (IFA and vitamin B12), the prevalence of serum vitamin B12 deficiency significantly decreased from 63.3% at baseline to 40.4% at post-intervention.

Participants in Group B had significantly better mean serum ferritin levels ($P = 0.035$) at post-intervention (18.2 ± 2.23 ng/ml to 36.6 ± 1.77 ng/ml) as compared with the IFA group (16.4 ± 2.80 ng/ml to 30.1 ± 2.03 ng/ml), indicating towards better improvement in iron stores in the vitamin B12-supplemented group. Similarly, Group B reported a significant ($P < 0.001$) increase in serum vitamin B12 levels from 156.0 ± 1.84 pg/ml at baseline to 219.2 ± 1.82 pg/ml at post-intervention (Table 5). Serum folic acid increased from 7.8 ± 3.31 ng/ml and 8.0 ± 3.31 ng/ml at baseline in Group A and Group B to 11.8 ± 3.65 ng/ml and 11.7 ± 3.58 ng/ml at post-intervention, respectively.

As serum ferritin is an acute phase reactant protein, whose value increases during infection, CRP was also analysed in a sub-sample of 268 participants (Group A: 138; Group B: 130) at post-intervention. The cutoff of > 3 mg/l was used. The overall mean CRP levels in the two groups were found to be similar, that is, 0.79 ± 1.60 mg/l (95% CI, 0.52–1.06) in Group A and 0.83 ± 1.84 mg/l (95% CI, 0.51–1.15) in Group B, respectively (*P*-value: 0.874).

Limitations of the study

The study was carried out at single site only and followed a weekly supplementation regime for 26 weeks. Multi-centre studies for longer duration are required to further assess the possible role of vitamin B12 supplementation on improving haemoglobin status and mechanism of iron storage in the body. In the study, serum vitamin B12 and serum folate levels were assessed to indicate deficiency among the study volunteers. However, the levels of surrogate biochemical markers that reflect the metabolic function

Table 4. Impact of supplementation in the two groups based on haemoglobin levels at baseline

Subjects with haemoglobin levels at baseline between (g/l)	*Group A (IFA+Placebo)*				*Group B (IFA+Cyanocobalamin)*			
	n	*Mean haemoglobin level at baseline (g/l)*	*Mean haemoglobin level at post-intervention (g/l)*	*% Increase*	n	*Mean haemoglobin level at baseline (g/l)*	*Mean haemoglobin level at post-intervention (g/l)*	*% Increase*
70–79	7	74.7	107.7	44.2	3	77.0	113.0	46.8
80–89	11	85.8	110.9	29.3	6	86.4	107.4	24.3
90–99	26	95.6	113.2	18.0	21	95.7	113.3	18.4
100–109	48	106.1	116.2	9.5	48	105.0	114.1	8.7
110–119	92	115.1	118.3	2.8	111	114.9	118.7	3.3

Abbreviations: IFA, iron folic acid; *n*, number of participants.

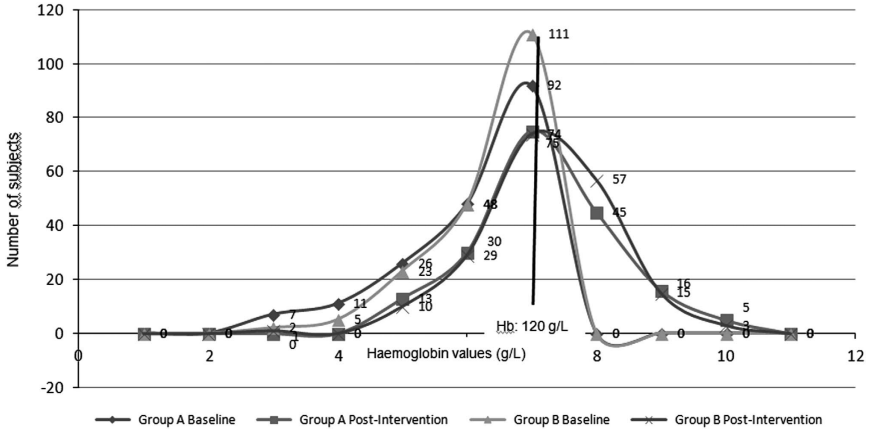

Figure 2. Haemoglobin curve before and after intervention in Group A and Group B.

Table 5. Mean levels of micronutrients before and after intervention

Parameters	*Group A (IFA+Placebo)* (n = *184*) *Mean levels*				*Group B (IFA+Cyanocobalamin)* (n = *189*) *Mean levels*				P-*value*[a]
	Baseline (BL)	*Post-intervention (PI)*	*Change (PI-BL)*	P-*value*	*Baseline (BL)*	*Post-intervention (PI)*	*Change (PI-BL)*	P-*value*	
Haemoglobin (g/l)	106.7 ± 11.2	116.4 ± 10.8	9.7 ± 13.7	< 0.001	108.9 ± 8.91	116.5 ± 10.26	7.5 ± 11.4	< 0.001	0.098
Mild anaemia (g/l)[b]	111.9 ± 5.08	117.6 ± 10.07	5.6 ± 10.64	< 0.001	111.9 ± 5.45	117.4 ± 9.06	5.4 ± 9.22	< 0.001	0.811
Moderate anaemia (g/l)[b]	89.8 ± 8.31	112.5 ± 12.25	22.7 ± 14.43	< 0.001	92.9 ± 6.07	112.1 ± 14.28	19.1 ± 14.74	< 0.001	0.304
S. Ferritin (ng/ml)	16.4 ± 2.80	30.1 ± 2.03	13.7 ± 21.59	< 0.001	18.2 ± 2.23	36.6 ± 1.77	18.0 ± 17.19	< 0.001	0.035
S. Folic acid (ng/ml)	7.8 ± 3.31	11.8 ± 3.65	3.9 ± 3.74	< 0.001	8.0 ± 3.31	11.7 ± 3.58	3.6 ± 4.18	< 0.001	0.350
S. Vit B12 (pg/ml)	152.9 ± 1.93	151.4 ± 1.93	0.95 ± 112.6	0.858	156.0 ± 1.84	219.2 ± 1.82	63.2 ± 141.69	< 0.001	< 0.001

Abbreviation: IFA, iron folic acid. [a]*P*-values signify the comparison between the change in mean values in Group A vs Group B. [b]Post-intervention mean levels among those who had mild or moderate anaemia at baseline (Group A: mild anaemia (*n* = 140); moderate anaemia (*n* = 44); Group B: mild anaemia (*n* = 159); moderate anaemia (*n* = 30).

of the vitamin like methylmalonic acid (MMA) for detection of vitamin B12 deficiency and plasma homocysteine for detection of either vitamin B12 or folate deficiency could not be undertaken.

DISCUSSION

The present study indicated that, with supervised IFA supplementation with or without vitamin B12, the mean haemoglobin levels of the participants increased by 9.7 ± 13.7 g/l and 7.5 ± 11.4 g/l in Group A (IFA group) and Group B (IFA and vitamin B12 group), respectively, and reduced the prevalence of anaemia by 37.9% (35.9% in Group A and 39.7% in Group B).

Other studies have also indicated a reduction in the prevalence of anaemia following weekly IFA supplementation, although the percent reduction in comparison with duration of supplementation is greater in the present study.[18–26] A study carried out among adolescent girls (12–19 years) in Vadodara, Gujarat (*n* = 2860), reported 21.5% and 10.3% reduction in the prevalence of anaemia (< 120 g/l) and serum ferritin deficiency (< 12 ng/ml), respectively, following 17 months of weekly IFA supplementation with a mean rise of haemoglobin levels by 6.4 g/l and serum ferritin levels by 4.8 ng/ml.[18] A study in urban slums, rural and tribal districts of Nashik, Maharastra, among adolescent girls aged 14–18 years (*n* = 1080) reported 11% reduction in the prevalence of anaemia following 30 months of weekly supplementation with 100 mg of iron and 500 mcg of folic acid.[19] A long-term study among school and non-school going adolescent girls in Lucknow, Uttar Pradesh, reported 48% reduction in the prevalence of anaemia after 4 years of weekly supplementation of IFA, family life education and deworming with an increase in haemoglobin levels from 10.8 to 11.8 g/100 ml.[20] Similarly, other authors have reported 10–25% reduction in the prevalence of anaemia following 1–6 months of daily or weekly IFA supplementation.[21–26]

Serum ferritin levels have also been reported to increase following supplementation of iron and folic acid. In a study that included Vietnamese adolescent girls, the prevalence of serum ferritin deficiency (< 15 µg/l) reduced from 14.0 to 4.5% at the end of 1 year of IFA supplementation with an increase in serum ferritin levels from 28.2 to 43.2 µg/l.[27] Similar results were observed by another group of investigators who studied Malaysian adolescent girls.[28] A rodent study concluded that cobalamin therapy normalised H-ferritin subunits in the spinal cord, suggesting that permanent cobalamin deficiency affects iron metabolism in the spinal cord.[29]

The role of vitamin B12 in reducing the prevalence of anaemia among adolescent girls has not been adequately explored under community settings. Scanty literature is available on the impact of vitamin B12 supplementation with iron and folic acid on pregnant women and children. In a recent randomised, placebo-controlled clinical trial to evaluate whether daily oral vitamin B12 supplementation during pregnancy (< 14 weeks of gestation in Bangalore, India) increases maternal and infant measures of vitamin B12 status, pregnant women were randomly assigned to receive daily oral supplementation with vitamin B12 (50 µg) (*n* = 183) or placebo (*n* = 183) through 6 weeks postpartum. Compared with placebo recipients, vitamin B12-supplemented women had significantly higher plasma vitamin B12 concentrations at both the second (median vitamin B12 concentration: 216 vs 111 pmol/l, $P < 0.001$) and third (median: 184 vs 105 pmol/l, $P < 0.001$) trimesters. However, no significant group differences in maternal MMA, total homocysteine (tHcy) or the prevalence of anaemia (haemoglobin < 11 g/100 ml) were noted at the second or third trimester time points.[30] Another randomised controlled trial in rural Querétaro, Mexico, to assess the response to a high-dose vitamin B12 supplementation to women 20–59 years concluded that supplementation increased serum vitamin B12 and holotranscobalamin and lowered MMA and tHcy. However, vitamin B12 supplementation did not affect haematology or bone-specific alkaline phosphatase.[31]

A study carried out among pregnant women (*n* = 800) attending antenatal clinic in Hyderabad, with haemoglobin less than 8.5 g/100 ml, showed that, after daily supplementation, the percent rise in haemoglobin was similar for the three groups, that is, elemental iron alone, iron and folic acid, and IFA and vitamin B12, whereas subjects in the control group showed a decrease in the haemoglobin level and concluded that no added haematological benefit was observed on giving folic acid and vitamin B12 supplementation with iron.[32] Another study among pregnant women (*n* = 112) in New Delhi also concluded that supplementation with 10 µg vitamin B12 daily for 4 weeks in addition to varied doses of iron and folic acid had no beneficial effect, although the group supplemented with folic acid in addition to iron showed a significant enhancement in the haemoglobin as compared with the group supplemented with only iron.[33] In another WHO-sponsored collaborative study on the effects of iron supplementation in pregnant women carried out in Delhi and Vellore, in which supplementation was given under supervision from the 26th week of pregnancy, groups receiving no iron showed a fall in mean haemoglobin concentration, whereas those receiving iron showed a rise in haemoglobin, the best results being in the groups receiving 120 and 240 mg of iron together with vitamin B12 and folate. Iron alone did not produce as good results as iron plus vitamin B12 and folate.[34]

In a group of Mexican preschoolers, oral supplementation with 1 mg/day vitamin B12 eliminated low serum concentrations of the vitamin, but had no effect on haemoglobin, mean cell volume or mean corpuscular haemoglobin concentration.[35] However, in a recent study conducted on 150 hospital-based children aged between 0.5 and 5 years, authors have concluded that the group supplemented with iron, folic acid and cyanocobalamin had higher haemoglobin level and percentage haemoglobin rise from baseline as compared with iron and folic acid alone.[36]

In the present study, vitamin B12 and folate levels were assessed as indicators of deficiency. However, both these assays have their limitations and may not reflect the true levels of the vitamins when used singly. The choice of test combinations depends on several considerations, including reliability, the setting in which the tests are used and the availability and cost of the tests.[37,38] For estimation of vitamin B12, currently available biomarkers can be categorised as those that directly measure the vitamin B12 in blood, that is, serum vitamin B12 and holotranscobalamin and those that measure metabolites, that is, MMA and tHcy that accumulate with inadequate amounts of vitamin B12.[39] A recent NHANES roundtable noted that serum vitamin B12 estimation reflects the broad vitamin B12 status ranging from high risk of severe deficiency to adequacy, whereas functional measures are useful for identifying subclinical vitamin B12 status and for reflecting early changes in vitamin B12 status. The roundtable further concluded that MMA is preferable to tHcy, because it increases with vitamin B12 inadequacy, but not with folate inadequacy, whereas both of these nutrient deficiencies affect tHcy. The roundtable concluded that, due to limitations with sensitivity and specificity of individual biomarkers, at least one biomarker of circulating vitamin B12 (serum vitamin B12 or holotranscobalamin) and one functional biomarker (MMA or tHcy) should be undertaken.[39]

A limitation of the plasma or serum folate concentration is that recent folate intake in a previously folate-deficient individual with normal absorptive capacity could result in an apparently normal plasma folate, and for population surveys to assess folate status the measurement of plasma or serum folate alone does not distinguish between a transient drop in folate intake and established chronic folate deficiency.[40] The NHANES roundtable also noted that red blood cell folate concentration is a better folate status measure, because it is an integrative measure of folate intake.[41]

The present study clearly indicates that the existing IFA dose and frequency regime recommended under National Iron Plus Initiative supplementation programme of Government of India can bring about a significant decrease in the prevalence of anaemia.

Furthermore, although IFA alone and IFA with vitamin B12 supplementation showed similar improvement with respect to haemoglobin levels and anaemia reduction, vitamin B12-supplemented group had significantly better serum vitamin B12 levels at post-intervention (63.3% at baseline to 40.4%) as compared with IFA group. Further, vitamin B12 group had better results in terms of reduced ferritin deficiency and improved ferritin levels. Hence, it is recommended that more multi-centric studies focussing on longer duration of supplementation or higher doses of vitamin B12 may be carried out.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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