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Treatment of anaemia in pregnancy with oral iron, folic acid or iron, folic acid and vitamin B12 supplementation: A hospital-based open randomized study

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ABSTRACT

Background and Aim: National iron plus initiative recommended that anaemic pregnant women should be treated with 200 mg of elemental iron and 1 mg folic acid. An increase in vitamin B12 deficiency has been reported in last two decades. An open randomised study was taken up in urban antenatal clinics to assess the impact of addition of vitamin B12 to the iron and folic acid supplementation.

Materials and Methods: Anaemic pregnant women willing to participate in the study were randomised into Group 1 (240 iron mg and 5 mg folic acid) or Group 2 (240 mg iron, 1.5 mg of folic acid and 15 μg vitamin B12); the impact of supplementation on Hb, ferritin, folic acid and vitamin B12 was assessed.

Results: In both groups mean Hb improved by 1g/dL at 8 weeks and by 1.5g/dL by 38 weeks of pregnancy; at 38 weeks only 30% were anaemic. Mean ferritin and folic acid levels improved in both groups. There was a fall in the mean vitamin B12 levels in the group which received 5 mg of folic acid.

Conclusion: With assured supply and supportive supervision, the supplementation achieved substantial reduction in anaemia in both groups. Supplementation with folic acid 5 mg should not be done because it causes a fall in vitamin B12 levels. The reason why addition of vitamin B12 to iron folic acid supplementation did not result in improvement in mean Hb, or vitamin B12 levels has to be investigated.

1. Introduction

The prevalence of anaemia in pregnancy in India is the highest in the world.[1,2] Anaemia in pregnancy is a major public health problem leading to high maternal morbidity and mortality, low birth weight and high infant mortality.[3–5] Over the last four decades there had been a decline in the prevalence of severe and moderate anaemia but the pace of decline is slow.[2] Major factors responsible for this were:

1. Accurate methods for Hb estimation were not available in primary and secondary care hospitals;
2. As anaemic women were not detected, all women were getting only prophylactic dose of iron folic acid supplementation;
3. Coverage of supplementation is not universal; and
4. Compliance with supplementation was low because of side effects associated with iron supplementation.[6]

These data were reviewed in 2013; the programme was renamed as, National Iron Plus Initiative (NIPI) and focussed on operationalising the 'test and treat' strategy.[7]

Studies from Pune[8] and Bangalore[9] indicate that in their study populations, folic acid deficiency was not high but vitamin (vit) B12 deficiency was widespread. Supplementation with folic acid in women who did not have folate deficiency but had vit B12 deficiency was associated

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with an increase in homocysteine levels and intrauterine growth retardation.[10,11]

Earlier iron folic acid supplements were centrally procured and provided to all the states. In the last two decades state governments had been procuring and supplying iron and folic acid tablets to antenatal clinics. Delhi Govt had been procuring and supplying 60 mg of iron and 5 mg of folic acid (IFA) tablets for ongoing IFA supplementation programme in antenatal clinics.[12] Obstetricians in many hospitals had been advising anaemic pregnant women to take two tablets each containing 60 mg of iron with breakfast and dinner and one tablet of folic acid 5 mg with lunch. There are no published studies assessing the impact of this treatment on haemoglobin (Hb) levels. Research studies published in the last two decades had reported a decline in folate deficiency and an increase in vit B12 deficiency. Taking this into account some obstetricians had been giving pregnant women vit B complex tablets containing 15 μg of vit B12 in addition to iron tablets. The impact of the addition of vit B12 to IFA supplements on Hb and vit B12 levels has not been investigated.

A hospital-based open randomised study was taken up to assess the impact of supplementation with 240 mg of iron and 5 mg of folic acid as compared to supplementation with iron 240 mg, folic acid 1.5 mg and vit B12 15 μg. The impact of supplementation for eight weeks on Hb, ferritin, folic acid and vit B12 levels was assessed. Data from the study will help to decide the dosage of iron and folic acid required for the treatment of anaemia and impact, if any, of addition of vit B12 on Hb and B12 levels.

2. Material and Methods

2.1. Study design

The study design is given in Figure 1. Hb estimation was done by cyanmethaemoglobin method in women attending the antenatal clinic early in the second trimester of pregnancy. Apparently healthy pregnant women with Hb levels between 8.0 and 10.9 g/dl who did not have any obstetric or systemic problems were given the study information sheet. The details about the study were explained to them. Three hundred and eleven women fulfilled the inclusion criteria and were willing to participate in the study. They were randomly allocated to:

1. Group 1 (152 women): those who took 2 tablets of ferrous sulphate each containing 60 mg of elemental iron with breakfast and 2 tablets of ferrous sulphate each containing 60 mg of elemental iron with dinner and 1 tablet of folic acid 5mg with lunch, or
2. Group 2 (159 women): those who took 2 tablets of ferrous sulphate each containing 60 mg of elemental iron with breakfast and 2 tablets of ferrous sulphate each containing 60 mg of elemental iron with dinner; 1 tablet of vit B complex (containing thiamine 10 mg, riboflavin 10 mg, niacinamide 100 mg, pyridoxine hydrochloride 3 mg, biotin 100μg, folic acid 1.5 mg, vit B12 15 μg, calcium pantothenate 50 mg and ascorbic acid 150 mg) with lunch.

At enrolment details of socio-demographic profile were obtained. Height (using wall-mounted stature meter; accuracy of 0.1cm) and weight (using digital weighing machine: accuracy of 100 g) and blood pressure (using a digital blood pressure monitor) were measured and obstetric examination findings were recorded in all women. Five ml of blood was drawn from venepuncture and samples were processed, and plasma was stored at -20°C till analysis.

Supplements for a fortnight were provided at enrolment. Women were given a form in which they were requested to record every day:

1. Whether they took or skipped the tablet;
2. If skipped, the reason for skipping;
3. Any side effect(s);
4. If yes, the nature, duration and severity of the side effect(s); and
5. Whether side effect(s) were seen with which tablets: iron folic acid or vit B12.

All women who continued to attend the antenatal clinic in the maternity centre received the supplements till delivery, according to the random allocation. These women were followed up every fortnight; compliance with supplementation was assessed using both the daily records kept by the woman and the number of tablets left in the tablet strip. Weight and blood pressure were measured and obstetric examination findings were recorded at every follow up visit. After 8 weeks of supplementation, and at 36-38 weeks of pregnancy blood samples were drawn, Hb estimated and the samples processed.

2.2. Sample size

Earlier studies had reported that IFA supplementation led to an improvement in Hb of about 1.0 g/dL[12]. Follow-up at 8 weeks was low (about 50%) because:

1. Families moved to new areas in search of jobs;
2. Women with obstetric problems referred to secondary care centres continued to have antenatal check-up in that hospital.

Sample size was calculated assuming that supplementation with iron 240 mg and one tablet of folic acid 5 mg/day is not inferior to supplementation with iron 240 mg, one tablet of vit B complex (containing folic acid 1.5 mg and vit B12 15 μg)/day; a difference of 0.5 g/dL in Hb levels will be a clinically significant difference; with α of 0.05 and β of 0.80, design effect of 2; the sample size was calculated to be 175. The sample size was computed to be 350 assuming a dropout rate of 50%.

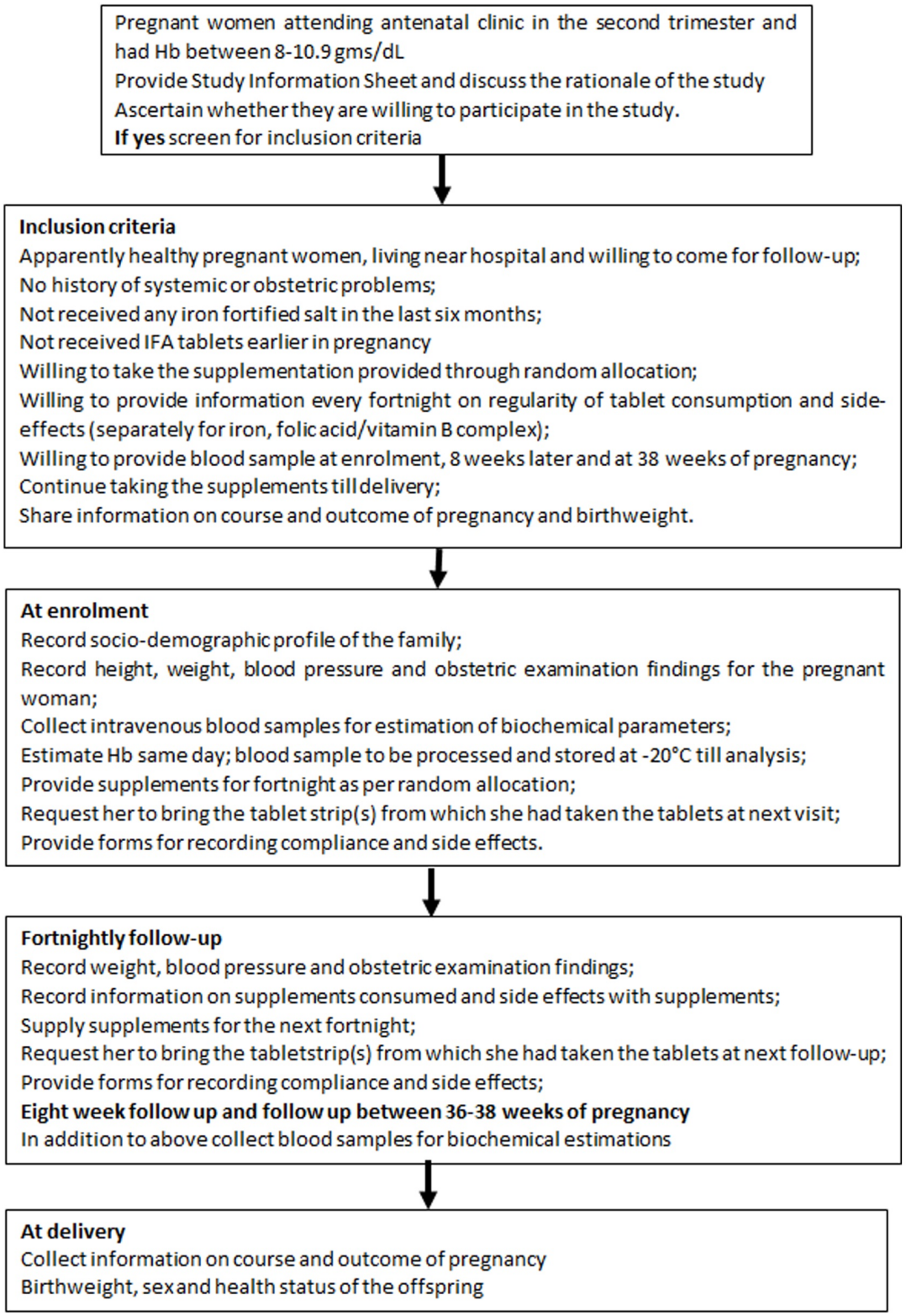

Figure 1: Study design

Directorate of Health Services, Delhi gave permission to conduct the study in the urban maternity hospitals. The Institutional Ethics Committee of Nutrition Foundation of India approved the study.

2.3. Biochemical estimations

Nutrition Foundation of India, estimated Hb by cyanmethaemoglobin method. National Institute of Nutrition, Hyderabad carried out all other biochemical assays. Ferritin assay was done using an in-house sandwich ELISA system with an assay range of 5-100 μg/L. Ferritin values had been corrected for inflammation using a factor 0.67 when C reactive protein (CRP) concentration was ≥5 mg/dL. A sandwich ELISA kit (R&D systems, Inc. MN, USA) was used for estimation of soluble transferrin receptor (sTfR). Human CRP assay kit (Alpha Diagnostic International, TX, USA) with a minimum detectable limit of 10 ng/mL, and assay range of 100-10,000 ng/mL was used for estimating CRP. A dual assay kit (Siemens Medical Solutions Diagnostics, Los Angeles, USA) was used for folic acid and vit B12 estimation. Hb estimation was done in all samples and ferritin assays were done in the majority of samples. CRP, sTfR, folate, vit B12 and glutathione peroxidase (GPx) assays were done in a sub-sample only. There were no differences in the profile of the sub-sample of women in whom sTfR, CRP, folate, vit B12 and GPx assays were carried out and the women in whose samples these assays could not be carried out.

2.4. Quality assurance

The ferritin levels were periodically cross-checked for quality in CDC VITAL-EQA program (VITAL-EQA program (laboratory number, 34). The quality control samples provided with the kits were used for checking the reliability of the other assays.

2.5. Data entry, data cleaning and analysis

MS excel was used for data entry and MS excel and SPSS were used for data cleaning. SPSS version 27 and Stata version 15 were used for data analysis.

Mean Hb, ferritin, CRP, sTfR, folic acid, vit B12 and GPx were computed. T test was used to assess statistical significance of changes in mean values across time in each group and inter-group differences between Group (Gr) 1 and Gr 2 at enrolment, after supplementation for 8 weeks and at 38 weeks of gestation; p values < 0.05 were considered as statistically significant.

Impact of supplementation on the prevalence of anaemia, iron, folic acid, and vit B12 deficiency was assessed by chi-square test in Gr 1 and Gr 2 in visit 1, 2 and 3; p values < 0.05 were considered as statistically significant.

3. Results

Table 1: Number of women at enrolment and follow up visits

	Group 1	Group 2
Enrolled	152	159
Follow-up 8 weeks	102	94
Follow-up at 38 weeks	29	19

A total of 152 women were enrolled for Gr 1 receiving 240 mg of elemental iron and 5 mg of folic acid and 159 women were enrolled for Gr 2 receiving 240 mg of elemental iron and one capsule of vit B complex containing 1.5 mg of folic acid and 15 μg of vit B12. Of these 102 women in Gr 1 and 94 women in Gr 2 continued the supplements for 8 weeks; follow-up samples were collected in them. Women who continued to attend the antenatal clinic in the same hospital were provided with supplements till delivery. A third sample was collected around 38 weeks of pregnancy in 29 women who continued taking the supplements in Gr 1 and 19 women in Gr 2 (Table 1).

Table 2: Socio-demographic profile

	Group 1 Fe 240 mg & folic acid 5mg (152)	Group 2 Fe 240 mg, folic acid 1.5mg & Vit B12 15 μg (159)
Type of family		
Joint	64.5	54.7
Nuclear	35.5	45.3
Size of family		
< 3	32.2	40.1
4-8	56.6	46.9
>8	11.2	13.0
Literacy of women		
Read and write	38.8	35.8
Had schooling	46.1	54.7
College	15.1	9.4
Literacy of husband		
Read & write	34.2	34.0
Had schooling	53.9	51.6
College	11.8	14.5
Work status of women		
Home maker	96.1	95.0
Working	3.9	5.0
Work status of husband		
Unskilled	4.6	1.3
Semi-skilled	65.8	61.0
Skilled	28.3	35.8
Standard of living index		
Low	61.8	52.8
Middle	37.5	46.5
High	0.7	0.6

Chi square test: None of the differences between Gr 1 and 2 were statistically significant

Table 2 gives the socio-demographic profile of the women enrolled for the study. The majority of the women were in their 20s and were literate home-makers belonging to low or low-middle-income groups. The majority of women and their husbands had school education; majority of men were employed as semi-skilled or skilled workers. There were no differences in the socio-demographic profile of the families at recruitment either between Gr 1 and Gr 2 or between those who discontinued and those who continued in either Gr 1 or Gr 2.

The obstetric profile of the women enrolled is given in Table 3. The majority of women were having their first or second pregnancies. None of these women had any obstetric problems because those with previous obstetric problems were not enrolled for the study. There were no differences in the obstetric profile at recruitment either between Gr 1 and Gr 2 or between those who discontinued and those who continued in either Gr 1 or Gr 2.

Table 3: Obstetric profile of women

	Group 1 Fe 240 mg & folic acid 5mg (152)	Group 2 Fe 240 mg, folic acid 1.5 mg & Vit B12 15 μg (159)
Gravida		
1	48.1	42.8
2	35.5	42.8
≥3	16.4	14.4
Parity		
0	52	43.4
1	35.5	44
2	11.2	10.7
≥3	1.3	1.9
Abortion		
0	91.5	94.3
1	5.9	4.4
≥2	2.6	1.3

Chi square test: None of the differences between Gr 1 and 2 were statistically significant

At enrolment the mean height was 151.2±3.32 cm in Gr 1 and 151.5±2.78 cm in Gr 2; the mean weight at recruitment was 49.9±7.47 kg in Gr 1 and 51.5±7.29 kg in Gr 2. There were no differences in the height and weight at the time of enrolment either between Gr 1 and Gr 2 or between those who discontinued and those who continued in either Gr 1 or Gr 2.

These data show that socio-demographic, nutrition and obstetric profile of Gr 1 and Gr 2 at enrolment were similar. There were no differences in the profile of the women who continued the supplementation and those who discontinued; therefore, it is likely that results from the follow-up group may apply to all the enrolled women.

3.1. Compliance with supplementation

In women who had continued taking the tablets for eight weeks and provided the second blood sample, the data on compliance with the supplementation regimen and side effects over 60 days were computed. Of 196 women (102 belonging to Gr 1 and 94 belonging to Gr 2) who took 240 mg of elemental iron/day 57% had reported abdominal discomfort, fullness, colic and/or constipation after taking iron tablets. They were reassured that these were known side effects of iron medication and were requested to continue iron tablets so that their Hb could improve. When the gastro-intestinal symptoms were persistent or severe women stopped taking iron tablets; when symptoms subsided, they resumed taking tablets. The mean number of days for which they had taken iron tablets was 39.5±6.4 in Gr 1 and 40.3±5.9 in Gr 2. There were no differences between Gr 1 and Gr 2 in the side effects or the number of days for which they took iron tablets in the 60-day period. The mean number of vit B complex tablets taken was 51.4±5.4; over a third of the women reported the smell of the tablets especially when they belched. The number of folic acid tablets taken was 55±1.67; women did not report any side effects with the folic acid tablets.

Mean values of biochemical parameters at 1^{st} (enrolment), 2^{nd} (8 weeks later) and 3^{rd} visit (at 38 weeks of pregnancy) in Gr 1 and 2 are given in Table 4. Paired mean values of biochemical parameters comparing the 1^{st} and 2^{nd} visits and 1^{st} and 3^{rd} visits in Gr 1 and 2 are given in Table 5. There were no differences between Gr 1 and Gr 2 in mean Hb, ferritin, sTfR, CRP, folic acid, vit B12, and GPx at enrolment. There was an increase in mean Hb by 1.0 g/dL after 8 weeks of supplementation and a further rise of 0.5 g/dL by 38 weeks of pregnancy in both Gr 1 and Gr 2; this improvement was statistically significant. At 8 weeks there was an increase in mean ferritin and reduction in sTfR both in Gr 1 and Gr 2. At 8 weeks the mean folic acid levels showed a statistically significant improvement in both groups; the magnitude of improvement was higher in Gr 1 who received 5 mg of folic acid/day. There was a fall in the mean vit B12 levels at 8 weeks in Gr 1 and no change in Gr 2. Mean GPx levels showed a statistically significant rise at 8 weeks which persisted at 38 weeks both in Gr 1 and Gr 2.

Prevalence of anaemia, iron deficiency at 8 weeks and at 38 weeks of pregnancy (paired samples) and folic acid and vit B12 deficiency at 8 weeks of supplementation is given in Table 6. In both groups prevalence of anaemia was reduced at 8 weeks; there was a further reduction in the prevalence of anaemia at 38 weeks. At 38 weeks only a third of women remained anaemic. The prevalence of folate deficiency was quite low at enrolment and virtually disappeared by 8 weeks of supplementation. Vit B12 deficiency was seen in over 40% of women both in Gr 1 and Gr 2. There was an increase in vit B12 deficiency at 8 weeks in women who

Table 4: Biochemical parameters at 1^{st}, 2^{nd} and 3^{rd} visit in Gr 1 and Gr 2

Biochemical parameter	Group 1 (Fe 240 mg& folic acid 5mg)		
	Visit 1 - Enrolment	**Visit 2 - 8 wk suppl**	**Visit 3 - 36-38 wk preg**
Hb (g/dL)	9.6±0.71 (150)	10.6±0.84 (95)	11.2±0.75 (29)
Ferritin (ng/ml)	23.2±34.83 (150)	28.6±37.09 (87)	29.6±28.50 (19)
sTfR (mg/L)	2.1±1.35 (149)	1.4±0.89 (49)	1.7±1.14 (19)
Folic acid (ng/ml)	8.5±6.87 (112)	14.5±11.94 (44)	-
Vit B12 (pg/ml)	232.4±138.31 (112)	214.9±138.77 (43)	-
GPx (μmol/min/gHb)	35.5±11.71 (113)	46.7±12.42 (50)	51.1±9.21 (19)
	Group 2 (Fe 240 mg, folic acid 1.5mg & Vit B12 15 μg)		
	Visit 1 - Enrolment	**Visit 2 - 8 wk suppl**	**Visit 3 - 36-38 wk preg**
Hb (g/dL)	9.5±0.71 (159)	10.6±0.87 (102)	11.4±0.65 (26)
Ferritin (ng/ml)	18.6±24.97 (159)	23.8±35.88 (92)	30.2±35.89 (18)
sTfR (mg/L)	2.1±1.15 (154)	1.3±0.75 (50)	1.6±0.84 (18)
Folic acid (ng/ml)	7.3±4.76 (115)	11.7±6.22 (46)	-
Vit B12 (pg/ml)	238.4±256.19 (117)	242.6±197.10 (46)	-
GPx (μmol/min/gHb)	36.0±12.19 (117)	48.6±14.62 (49)	43.9±17.11 (18)

Comparison across period of use student t test p value
Gr 1 Visit 2 and 1: Hb 0.0000; folate 0.0010; sTfR 0.0004; GPx 0.0000.
Gr 1 Visit 3 and 1: Hb 0.0000; GPx 0.0000
Gr 2 Visit 2 and 1: Hb 0.0000; folate 0.0000; sTfR 0.0000; GPx 0.0000.
Gr 2 Visit 3 and 1: Hb 0.0000; ferritin 0.0385; sTfR 0.0378; GPx 0.0086.
Inter group comparison student t test p value
None significant

Table 5: Biochemical parameters at 1^{st} and $2^{nd}$and 1^{st} and 3^{rd} visits (paired samples) in Gr 1 & 2

Biochemical parameter		**Group 1 (Fe 240 mg& folic acid 5mg)**		
		Visit 1	**Visit 2**	**Visit 3**
Hb (g/dL)	visit 2 vs 1	9.5±0.70 (95)	10.6±0.84 (95)	
	visit 3 vs 1	9.8±0.53 (29)		11.2±0.75 (29)
Ferritin (ng/ml)	visit 2 vs 1	29.2±43.73 (87)	28.6±37.09 (87)	
	visit 3 vs 1	15.9±16.58 (19)		29.6±28.50 (19)
sTfR (mg/L)	visit 2 vs 1	2.0±1.31 (49)	1.4±0.89 (49)	
	visit 3 vs 1	2.3±1.64 (19)		1.7±1.14 (19)
Folic acid (ng/ml)	visit 2 vs 1	7.7±5.25 (44)	14.5±11.94 (44)	
Vit B12 (pg/ml)	visit 2 vs 1	243.3±151.30 (43)	214.9±138.77 (43)	
GPx (μmol/min/gHb)	visit 2 vs 1	33.0±12.45 (50)	46.7±12.42 (50)	
	visit 3 vs 1	33.2±10.37 (19)		51.1±9.21 (19)
Biochemical parameter		**Group 2 (Fe 240 mg, folic acid 1.5mg and Vit B12 15 μg)**		
		Visit 1	**Visit 2**	**Visit 3**
Hb (g/dL)	visit 2 vs 1	9.5±0.70 (102)	10.6±0.87 (102)	
	visit 3 vs 1	9.6±0.57 (26)		11.4±0.65 (26)
Ferritin (ng/ml)	visit 2 vs 1	19.9±29.12 (92)	23.8±35.88 (92)	
	visit 3 vs 1	16.5±20.04 (18)		30.2±35.89 (18)
sTfR (mg/L)	visit 2 vs 1	2.2±1.24 (50)	1.3±0.75 (50)	
	visit 3 vs 1	2.5±1.08 (18)		1.6±0.84 (18)
Folic acid (ng/ml)	visit 2 vs 1	7.0±4.88 (44)	11.5±6.18 (44)	
Vit B12 (pg/ml)	visit 2 vs 1	282.1±374.84 (46)	242.6±197.1 (46)	
GPx (μmol/min/gHb)	visit 2 vs 1	32.6±9.34 (49)	48.6±14.62 (49)	
	visit 3 vs 1	32.1±7.27 (18)		43.9±17.11 (18)

Comparison across period of use paired t test single tailed
Gr 1 Visit 2 and 1: Hb 0.0000; folic acid 0.0004; GPx 0.0000; sTfR 0.0047.
Gr 1 Visit 3 and 1: Hb 0.0000; ferritin 0.039; GPx 0.0000
Gr 2 Visit 2 and 1: Hb 0.0000; folate 0.0001; GPx 0.0000; sTfR 0.0000;
Gr 2 Visit 3 and 1: Hb 0.0000; GPx 0.0055; sTfR 0.004;
Inter group comparison Student 't' test
None significant

had received folic acid 5mg and no change in prevalence of B12 deficiency in Gr 2 who had received both folic acid and vit B12 (Table 6).

4. Discussion

National iron plus initiative (NIPI) 7 emphasised that:

1. All pregnant women should be screened for anaemia using accurate test for Hb;
2. Non-anaemic women should get one tablet of IFA (100 mg of elemental iron and 500 μg of folic acid) per day and anaemic women should get 2 tablets of IFA every day;
3. IFA tablets should be taken after meals so that side-effects are minimised; and
4. Improvement in Hb should be monitored in anaemic women and those not responding to supplementation should be referred to secondary care centres for investigation and management.

In the present study screening of anaemic pregnant women attending antenatal clinics in urban primary health care institutions was operationalised and at the initial screening anaemic women with Hb between 8.0 and 10.9 g/dL were identified. Delhi government had been procuring and providing 60 mg iron tablets and 5mg folic acid tablets to antenatal clinics in government hospitals. Obstetricians in these antenatal clinics requested anaemic women to take two tablets of iron each after breakfast and dinner and one tablet of folic acid after lunch. In the present study, women in Gr 1 followed this practice; women in Gr 2 took two tablets of iron each after breakfast and dinner and one tablet of vitamin B complex after lunch.

Prevalence of side-effects and compliance with the supplementation regimen over 60 days of supplementation were computed in women who had continued taking the tablets for eight weeks and provided the second blood sample. Nearly 60% of women taking 240 mg of elemental iron/day reported abdominal discomfort, fullness, colic and/or constipation. In our earlier study on supplementation with elemental iron 60 mg/day side effects were reported in about 40% of women.[12] In women supplemented with 240 mg iron, side-effects often persisted throughout the day. Women were reassured that this was a known problem with iron medication and they had to continue iron tablets to improve their Hb. When the gastro-intestinal symptoms were persistent or severe, women stopped taking iron tablets for a few days; when symptoms subsided, they resumed taking tablets. Of the 60 days from enrolment to 1^{st} follow-up visit, the mean number of days for which they had taken iron tablets was 39.5±6.4 in Gr 1 and 40.3±5.9 in Gr 2. There were no differences in the side effects or the number of days for which they took iron tablets in the 60 days between Gr 1 and Gr 2.

All research studies and surveys indicate that even in pregnant women the continued intake of IFA supplementation was relatively low. There had been suggestions that women forgetting to take the tablets could be a factor responsible for the poor compliance with IFA supplementation. In the present study, the importance of continued intake of the supplements was emphasized and compliance with iron, folic acid and vitamin B complex was checked separately at each visit.

The mean number of vit B complex tablets taken was 51.4±5.4; over a third of the women reported the smell of the tablets especially when they belched. The number of folic acid tablets taken was 55±1.67; women taking the folic acid tablets did not report any side effects. Data from the present study showed that under research conditions:

1. Compliance and tablet intake were highest with folic acid tablets which was not associated with any side effects;
2. Compliance and tablet intake were lower with vit B complex tablets; there were no side-effects reported with vit B complex, but some of the women did not like the smell of the tablets;
3. Compliance and tablet intake were lowest with iron tablets. Compliance computed for the 60 days showed that women took the iron tablets only for about 40 days because over half of the women had side effects with iron supplementation.

These data suggest that side effects were the major reason for lower compliance with iron supplementation. Pregnant women do remember to take tablets after meals and try to comply with supplementation because they want their child to be born healthy. With intensive nutrition education and supportive supervision, it was possible to improve compliance with supplementation during pregnancy to about 65%.

Data from the present study and the earlier studies[6,12] indicate that side effects of iron play a major role in poor compliance and continuation rates with iron supplementation. In recent years the dose of iron required for correction of anaemia has been extensively investigated and efforts have been made to assess the minimal effective dose for iron supplementation. Studies carried out in India had shown that 60 mg of elemental iron in IFA supplementation was adequate to bring about improvement in anaemia in over two-thirds of anaemic pregnant women.[12,13] Comparison of data from the present study with our earlier study showed that lower dosage of iron is associated with lower prevalence of side effects.

Our institution had earlier undertaken a hospital-based open randomised study to find out the impact of supplementation with 60 mg of elemental iron with either 5 mg folic acid or one tablet of vit B complex containing 1.5.mg of folic acid and 15 μg of vitamin B 12. Comparison

Table 6: Prevalence of biomarker deficiency in Gr 1 and Gr 2 at visit 1, 2 and 3

Parameter	Visits	Group 1 (Fe 240 mg& folic acid 5mg)			Group 2 (Fe 240 mg, folic acid 1.5mg and Vit B12 15 μg)		
		Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
Hb (g/dL)	visit 2 vs 1	100.0	62.1		100.0	61.8	
	visit 3 vs 1	100.0		31.0	100.0		26.9
Ferritin (ng/ml)	visit 2 vs 1	47.1	24.1		52.2	38.0	
	visit 3 vs 1	68.4		36.8	77.8		27.8
sTfR (mg/L)	visit 2 vs 1	79.6	85.7		68.0	90.0	
	visit 3 vs 1	73.7		73.7	61.1		83.3
Folic acid (ng/ml)	visit 2 vs 1	4.5	0.0		19.0	2.4	
Vit B12 (pg/ml)	visit 2 vs 1	40.9	58.1		50.0	50.0	

Comparison across period of use chi square test p value
Gr 1 visit 2 & 1: Hb <0.000; ferritin 0.002;
Gr 1 visit 3 & 1: Hb <0.000; ferritin 0.05.
Gr 2 visit 2 & 1: Hb <0.000; ferritin 0.05; FA 0.01; sTfR 0.01.
Gr 2 visit 3 & 1: Hb <0.000; ferritin 0.003.

Table 7: Difference (mean±SD) in Hb and ferritin (paired values between visits) in those who had iron 60mg and iron 240mg in Gr 1 and Gr 2

	Iron 60 mg		Iron 240 mg	
	Group 1 Fe 60 mg & folic acid 5mg	Group 2 Fe 60 mg, folic acid 1.5mg & Vit B12 15 μg	Group 1 Fe 240 mg & folic acid 5mg	Group 2 Fe 240 mg, folic acid 1.5mg & Vit B12 15 μg
Hb 2 & Hb 1	1.16±1.099 (68)	1.09±1.054 (91)	1.00±0.731 (95)	1.09±0.754 (102)
Hb 3 & Hb 1	1.79±1.267 (20)	1.65±1.221 (30)	1.42±0.726 (29)	1.72±0.829 (26)
Ferritin 2 vs 1	4.64±13.01 (66)	6.02±34.283 (85)	-0.60±36.94 (87)	3.95±29.362 (92)
Ferritin 3 vs 1	7.54±17.931 (27)	3.03±23.794 (37)	13.69±22.903 (19)	13.71±40.475 (18)

Comparison of mean & SD of differences in Fe 60mg and Fe 240 mg in both groups by 'T' test
The differences in mean Hb and ferritin between women who received 240mg and 60mg in visit 1, 2, and 3 in Group 1 and Group 2: None were statistically significant

of the data on mean Hb and ferritin levels in the earlier study with the data from the present study with 240 mg of elemental iron with either one tablet of 5mg folic acid or one tablet of vit B complex supplementation is given in Table 7. There were no differences in the improvement in the mean Hb or ferritin in visit 2 or 3 between the group of women who received 60 mg of elemental iron and those who received 240 mg of elemental iron either in Gr 1 or Gr 2.

With either dose about 70% of women became non-anaemic (Hb ≥11g/dL) at 38 weeks of pregnancy. These data suggest that 240 mg of iron/day is not required for treatment of anaemia in pregnancy.

Concern had been expressed that even under research conditions one-third of the pregnant women receiving IFA remain anaemic. Not all anaemia is due to iron deficiency. Some of the available data suggest that only about two-thirds of all anaemic women have iron deficiency.[12–14] Only iron deficient pregnant women will respond to iron therapy. Anaemic women with haemoglobinopathies or infections like malaria will not respond to IFA therapy. It is therefore likely that the expected reduction in the prevalence of anaemia in response to iron supplementation will be around 70%. It is therefore essential that all anaemic women on IFA supplementation be monitored using an accurate test for Hb for improvement in Hb over the next two months; all non-responders should be identified and sent to secondary/tertiary care centres for investigation and treatment.

Studies from other countries had shown that divided doses of iron/day may not be associated with increase in absorption or utilisation of iron.[15,16] There had been reports that excess unabsorbed iron from oral iron supplements can adversely affect the gut microbiota.[17,18] In view of these observations, it is essential to ensure that the dose of iron supplementation is kept at the minimum effective level and is administered once a day after a meal.

Data from the present study and other studies in India[8,9] have shown that over time there had been a reduction in the prevalence of folic acid deficiency and rise in prevalence of vit B12 deficiency. In the present study at enrolment, prevalence of folic acid deficiency was very low and vit B12 deficiency was seen in 40-50% of women. In Delhi hospitals women received 5 mg of folic acid

supplementation. Compliance and continuation rates with folic acid supplementation was high because folic acid supplementation is not associated with any side effects. Sustained supplementation of 5 mg of folic acid resulted in improvement in mean folate levels but resulted in fall in vit B12 levels. Increase in already high vit B12 deficiency in pregnancy may have adverse health consequences[8–10] and therefore folic acid supplementation at 5mg level should be avoided. Supplementation with vit B12 15 μg and folic acid 1.5 mg resulted in improvement in mean folic acid level but did not result in higher mean vit B12 levels or higher mean Hb levels as compared to those who received IFA. Similar results had been reported in the 1970s[19,20] and in our earlier study.[12] The reasons for non-response in Hb levels and vit B12 levels following oral vitamin B12 supplementation have to be investigated.

In 2018 the progress in anaemia control programme was reviewed and it was recommended that the programmes should be intensified. The Intensified National Iron Plus initiative (I-NIPI)[21] recommended that:

1. All pregnant women should be screened by an accurate method for Hb estimation;
2. Non anaemic women should receive one tablet of 60 mg of elemental iron and 500μg of folic acid per day for preventing anaemia; iron tablet should be taken after a meal;
3. Anaemic women should receive 2 tablets of 60 mg of elemental iron and 500μg of folic acid per day for treatment of anaemia; both tablets should be taken together after one meal;
4. Improvement in Hb in anaemic women should be monitored;
5. Women not showing improvement in Hb with IFA supplementation should be referred to secondary/tertiary care hospitals for investigation and management.

   I NIPI emphasised the need for increasing iron intake and Hb status before pregnancy through dietary diversification and use of iron-fortified food stuffs such as salt prior to pregnancy, and IFA supplementation to women in reproductive age. Effective implementation of the multi-pronged I-NIPI strategy is expected to accelerate the reduction in prevalence of anaemia.[22]

5. Conclusion

An open randomised study hospital-based in anaemic pregnant women (Hb 8.0 to10.9 g/dL) in their second trimester was undertaken to assess the impact of supplementation, with 240 mg iron with either folic acid 5mg (Gr 1) or vit B complex containing folic acid 1.5mg and vit B12 15μg (Gr 2) for 8 weeks, on Hb and biochemical parameters.

In both the groups mean Hb showed an improvement by 1g/dL at 8 weeks; the prevalence of anaemia was reduced by one-third. In the small group of women who continued treatment till 38 weeks there was a further improvement of 0.5 g/dL in the mean Hb and reduction in prevalence of anaemia to 30% in both the groups. Mean ferritin and folic acid levels improved in both groups. There was no difference in mean Hb, prevalence of anaemia, mean ferritin or folic acid between Gr 1 and Gr 2. There was a fall in the mean vit B12 levels in the group which received 5mg of folic acid. The addition of vit B12 did not result in improvement in mean Hb or vit B12 levels. Comparison of data on mean Hb and ferritin following supplementation with 60 mg of elemental iron or 240 mg of elemental iron showed that there were no differences in the mean Hb and ferritin between the two doses of iron; side effects were more with 240 mg supplementation.

With assured supply and supportive supervision, it was possible to achieve good compliance with supplementation and substantial reduction in anaemia in both groups receiving 240 mg of elemental iron, despite troublesome minor gastro intestinal disturbances experienced by 2/3rd of women. Data from earlier study showed that lower dose of iron was associated with lower prevalence of side effects and that similar improvement in Hb can be achieved with lower dose of iron. Supplementation with folic acid 5 mg should not be done because it causes a fall in vitamin B12 levels. The reason why addition of vitamin B12 to iron folic acid supplementation did not result in improvement in mean Hb, or vitamin B12 levels has to be investigated.

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7. Conflict of Interest

None.

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