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ORIGINAL ARTICLE

Weekly iron folic acid supplementation plays differential role in maintaining iron markers level in non-anaemic and anaemic primigravida: A randomized controlled study

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Abstract Anaemia during pregnancy is most commonly observed and highly prevalent in South-East Asia. Various effective programmes have been laid down for its management, mainly daily supplementation of iron folic acid (IFA) tablets. Following the same, standard obstetrical practice has included the IFA supplementation without requiring the determination of iron deficiency. In this study, a total of 120 primigravida ($N = 60$; non-anaemic (Hb > 11 g/dl) and $N = 60$ anaemic (Hb = 8–11 g/dl)) were selected among those attending the Antenatal Clinic in Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India. They were supplemented with daily and weekly IFA tablets till 6 weeks postpartum. Corresponding changes in haemoglobin level on advance of pregnancy, side effects and compliance

Abbreviations: DISG, daily IFA supplementation group; Hct, haematocrit; Hb, haemoglobin; HsCRP, high sensitivity C-reactive protein; IFA, iron folic acid; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; RBC, red blood cell; sTfR, soluble transferrin receptors; WISG, weekly IFA supplementation group

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associated with daily and weekly IFA supplementation and its associations with iron status markers were studied. The inflammatory markers were also estimated. The statistical significance level ($p < 0.05$) between the groups were assessed by applying unpaired t-test using SPSS (version 16.0). The obtained results publicized the salutary role of daily IFA supplementation in improving the haemoglobin level and iron status markers in anaemic pregnant women though the levels could not reach up to the non-anaemic haemoglobin levels. However, weekly IFA supplementation seems to be a better approach in non-anaemic pregnant women where almost comparable results were obtained in terms of haematological parameters, gestation length and birth weight.

Conclusion: Weekly IFA supplementation found to be as effective as daily supplementation in iron sufficient non-anaemic pregnant women whereas anaemic pregnant women should be prescribed daily IFA supplementation irrespective of iron replete/deplete state.

1. Introduction

Almost all over the world daily oral iron supplementation is routinely prescribed during the pregnancy irrespective of deplete or replete iron status at pre-conception or post-conception and the average dose range is 60–120 mg supplemental iron per day as recommended by The International Anaemia Consultative Group (Stoltzfus and Dreyfuss, 1999). The upper dose range is recommended where there is a high prevalence of anaemia among pregnant women. In India, anaemia is highly prevalent in pregnant as well as non-pregnant women (>50% are anaemic) (De Benoist et al., 2008) that resulted in 40% of maternal deaths (Kalaivani, 2009).

Iron deficiency is one of the underlying causes of anaemia during pregnancy (De Benoist et al., 2008) and is one of the 15 leading contributors to the global burden of disease (WHO and CDCP, 2004). Regulation of iron is a highly sophisticated phenomenon where its imbalance could result into significant morbidity and mortality. The disturbed intricate balance during iron regulation may either result in iron deficiency or iron overload, out of which iron deficiency is most commonly observed (Zimmermann and Hurrell, 2007). Iron deficiency anaemia is the most common nutritional disorder prevalent both in developed and developing countries particularly in pregnant women of developing countries (WHO, 2001). This is due to iron deficit intake of diet that could not meet the increased iron demand for the developing foetus (Zimmermann and Hurrell, 2007). According to World Health Organization (WHO, 2001) around 2 billion people who count approximately 30% of the world population is anaemic; pregnant women contributes approximately 41.8% of this anaemic polulation (De Benoist et al., 2008).

The most common strategy for the management of this staggering situation is to start oral iron supplementation during pregnancy. In India, a National Nutrition Policy was adopted in 1993 to reduce prevalence of anaemia and the supplementation interventions include daily supplementation of $FeSO_4$ containing 100 mg elemental iron tablet and 500 μg folic acid for 100 days during pregnancy followed by the same dose for 100 days in the postpartum period (Guidelines for control of iron deficiency anaemia, 2013).

During the advance of pregnancy, plasma volume expands resulting in the decreased haematocrit and Hb levels resulting in the fluctuations in the values. Thus, the reliability of Hb measurement and haematocrit diminished (Bentley, 1985). Ferritin level, an indicator of iron storage decreased during the gestation but its level was found elevated in inflammatory conditions, being an acute phase reactant. Thus, measurement of sTfR along with these parameters would help to detect iron deficiency state more precisely because neither is it an acute phase reactant (Skikne, 1998) nor influenced by hemodilution during pregnancy (Akinsooto et al., 2001). The inflammatory markers like cortisol and high sensitivity C-reactive protein were also studied to ensure the changes in the level of iron markers were true reflection of oral iron supplementation and not influenced by inflammation. Erythropoietin hormone responsible for the expansion of red cell mass whose level continuously increased during the pregnancy that led to the rise in Hb concentration (Barton et al., 1994; McMullin et al., 2003; Milman et al., 1997). Thus measurement of aforementioned markers could reflect the complete iron profile of the body.

Although, the effect of daily versus weekly IFA supplementation on iron status markers during pregnancy has been studied scanty information is available about the amount and regularity of IFA intake in comparison with iron status markers level and also the side-effects associated with different supplementation schemes. Owing to these, the present study was designed to examine the impact of daily versus weekly oral iron supplementation in improving the iron status makers, Hb levels, birth weight and effect on erythropoietin level, gestation length and mode of delivery in anaemic/non-anaemic pregnant women during gestation and postpartum period.

2. Material and methods

2.1. Subjects

The present study comprised a total of 120 pregnant women in their early second trimester (13–16 weeks of gestation) who are yet to start oral iron supplementation. Out of 120, 60 were non-anaemic (Hb > 11 g/dl) women and 60 were anaemic (Hb = 8–11 g/dl) women. The subjects were selected among those attending the Antenatal Clinic in Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India. Pregnant women fulfilling the subject selection criteria, having no chronic morbidity, 20–30 years old primigravida, and belong to middle socio-economic group willing to participate (informed consent obtained) were enrolled in the study. Exclusion criteria include

history of any metabolic disease like diabetes mellitus, malignancy and heart disease, infectious diseases like tuberculosis, HIV, endocrine disorders or taking any iron preparations during or from past 3 months.

2.2. Randomization

The participants fulfilling the screening criteria were allocated to anaemic group on the basis of Hb range between 8–11 g/dl and non-anaemic group (Hb > 11 g/dl). After informed consent process, envelopes containing random number slips for 60 pregnant women in daily and 60 pregnant women in weekly arm prepared at Indian Council of Medical Research provided to the participating centre were opened for allocation in daily and weekly arm. Random numbers were generated in a ratio of 1/1 by means of a customized computer programme. According to the serial number of the enrolled subject, one sealed envelope containing a slip with instructions for random allocation of subject to either daily or weekly arm was opened for each subject. The Participants were assigned the iron supplement regimen accordingly. The enrolled participants were categorized into two groups as follows:

Group 1: pregnant non-anaemic (Hb > 11 g/dl) women ($N = 60$).
Group 2: pregnant anaemic (Hb = 8–11 g/dl) women ($N = 60$).

Informed consent was obtained from each subject and the study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, New Delhi, India.

2.3. Study design

All the enrolled participants (anaemic and non-anaemic pregnant women) were dewormed by giving single dose of Albendazole 400 mg to be consumed at night on the day of enrolment. The enrolled participants were asked to fast overnight and on the next morning venous blood sample was drawn from anticubital vein in supine position followed by refreshments. Likewise, second sample (3 months after first sampling) and third sample (6 weeks postpartum) were obtained in a similar manner.

According to random allocation of slip instructions, participants were given either one tablet of $FeSO_4$ containing 100 mg elemental iron and 500 μg folic acid (iron folic acid tablet/IFA tablet) till 6 weeks postpartum daily or two IFA tablets weekly till 6 weeks postpartum. Thus, both the groups i.e. group 1 and 2 further subdivided into daily IFA supplementation group (DISG; $N = 30$) and weekly IFA supplementation group (WISG; $N = 30$). There was no placebo group in the study as iron deficiency is widely prevalent and denying iron to any participant was not possible since daily iron supplementation is included in the Indian National Programme.

2.4. Sample collection

6 ml venous blood was taken from each participant and serum separated by centrifuging the blood in clot activator tubes at 3000 rpm for 15 min. Different aliquots were prepared and stored in −80 °C till further analysis.

2.5. Analytical estimation

2.5.1. Measurement of blood index parameters

Blood haemoglobin (Hb) was determined by using the Cyanomethemoglobin method (Cook, 1985). Haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell (RBC) counts were determined by using Sysmax A-380 automated cell counter.

2.5.2. Measurement of iron and inflammatory markers

Serum was analyzed for ferritin, soluble transferrin receptors (sTfR), erythropoietin, cortisol and high sensitivity C-reactive protein (HsCRP) and levels were measured using competitive ELISA kits. The results were compared with those of the standard curve obtained from the calibrators run simultaneously with the study samples for each parameter. Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) (JY 2000 2, Jobin Horiba) technique was used for measuring the serum concentration of iron.

2.6. Statistical analysis

Data were expressed as mean ± SE. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0. In order to evaluate significance level between the groups, unpaired *t*-test was applied and the statistical significance level ($p < 0.05$) was also determined using a one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Repeated measure ANOVA was also applied to check the level of significance as each subject was measured for a particular variable at three time points.

3. Results and discussion

The present study comprised non-anaemic and anaemic primigravida with age group between 19–30 years, BMI ranged 18–22 kg/m^2, belonging to middle socio-economic status between 11 and 25 on Kuppuswamy's socioeconomic status scale.

3.1. Daily versus weekly IFA supplementation in non-anaemic pregnant women

3.1.1. Amount and regularity of IFA tablets intake from baseline to second visit

The results of the study showed that the mean number of IFA tablets intake was 88.93 in DISG compared to 24.32 in WISG. The regularity of IFA tablets intake found to be 100% as highly regular in WISG compared to DISG where 89.2% showed <3 IFA tablets intake defaults and 10.7% were irregular. Although the symptoms due to IFA tablets intake were more commonly observed in WISG compared to DISG but the severity of the symptoms was mild. The percentage of women who withheld IFA due to side effects was 2.6-fold high in DISG with comparatively low percentage of women who withheld IFA due to forgetfulness than WISG (Table 1).

3.1.2. Amount and regularity of IFA tablets intake from second visit to 6 weeks postpartum

The mean IFA tablets intake was found to be 124.7 in DISG as compared to 33.7 in WISG where 95.8% women were highly

Table 1 Amount, regularity and symptoms of iron folic acid tablets (IFA) intake.

IFA supplementation groups		Number of IFA tablets consumed (Mean ± SE)	Regularity of IFA tablets consumed	Subjects not taken IFA due to side effect (number of times)	Subjects not taken IFA due to forgetfulness (number of times)	Symptoms due to IFA intake- subjects	Severity
Non-anaemic WISG	Baseline to second visit	24.32 ± 0.29	100%	4% (one time)	12% (one time)	Nausea 4% Vomiting 16% Abdomen pain 8% Headache-4%	Mild Mild Mild Mild
	Second visit till 6 weeks PP	33.79 ± 1.01	95.8% 4.1%	8.3% (50% two times and 59% four times)	16.6% (two times)	Vomiting-16.6% Constipation-4.1%	Mild Mild
Non-anaemic DISG	Baseline to second visit	88.93 ± 0.89	89.2% 10.7%	10.7% (66.6% one time and 33.3% two times)	7.1% (50% one time and 50% for two times)	Vomiting-7.1% Constipation-3.5% Loss of appetite-3.5%	Mild Mild NIL
	Second visit till 6 weeks PP	124.7 ± 2.47	88.0% 12.0%	4.0% (one time)	20.0% (40% three times and 20% nine times, 20% ten times and 20% fifteen times)	Constipation-4%	Mild
Anaemic WISG	Baseline to second visit	24.20 ± 0.35	100%	NIL	NIL	Distention abdomen-10% Constipation-10% Vomiting-10%	Mild Moderate Mild
	Second visit till 6 weeks PP	31.60 ± 3.57	62.5%[1] 25%[2] 12.5%[3]	12.5% (six times)	37.5% (33.3% one time, 33.3% five times and 33.3% seven times)	Nausea-12.5% cases Vomiting-12.5%	Mild Moderate
Anaemic DISG	Baseline to second visit	89.44 ± 1.35	77.7% 22.2%	NIL	22.2% (50% five times and 50% six times)	Acidity-11.1%	NIL
	Second visit till 6 weeks PP	116.3 ± 4.37	66.6% 22.2% 11.1%	11.1% (30 times)	55.5% (40% two times and 20% three times, 20% five times and 20% seven times)	Fever-11.1%	Moderate

WISG – weekly IFA supplementation group; DISG – daily IFA supplementation group; PP – postpartum.
[1] Highly regular < 3 IFA tablets intake defaults.
[2] Irregular = 3–7 IFA tablets intake defaults.
[3] Highly irregular > 7 IFA tablets intake defaults.

regular and 4.1% irregular compared to 88% highly regular and 12% highly irregular in DISG. The percentage of women who withheld tablets due to side effects was more than double in WISG (8.3% versus 4%) whereas this group comprised comparatively less percentage of women who withheld the IFA tablets due to forgetfulness (16.6% versus 20%). The percentage of women with symptoms like mild vomiting and constipation was observed more in WISG than DISG (Table 1).

3.1.3. Haematological markers and delivery outcomes

All the haematological parameters (Hct, RBC count, MCV and MCH) found comparable in both the supplementation groups, however statistically significant difference was observed while analyzing these parameters using repeated measure ANOVA in DISG {(Hb: F (2,48) = 7.78; p = 0.001); (Hct: F (2,44) = 8.23; p = 0.001); (RBC count: F (2,46) = 21.53; p = 0.000); (MCV: F (2,44) = 3.761; p = 0.035); (MCH: F (2,46) = 4.92; p = 0.012)} and WISG {(Hb: F (2,50) = 7.77; p = 0.001); (Hct: F (2,50) = 8.90; p = 0.000); (RBC count: F (2,50) = 14.43; p = 0.000); (MCH: F (2,50) = 7.90; p = 0.001)}. In addition there was no significant difference observed in gestation length or birth weight (Table 2). However, the percentage of women underwent caesarean delivery was found to be high in WISG compared to DISG (48% versus 28.5% i.e. 1.68-fold high) and that group was also found to have significantly low Hb at postpartum compared to DISG ($p < 0.05$).

3.1.4. Iron status markers

The effect of daily and weekly oral IFA supplementation is summarized in Table 3 where the non-significant difference was observed in most of the studied parameters. The significant difference was obtained in haemoglobin measured at postpartum in DISG ($p < 0.05$) which is in relation with the serum levels of ferritin ($p < 0.01$) and iron ($p < 0.05$) as compared to WISG. However, a significant increase in serum level of iron was found in DISG at second visit also ($p < 0.01$). DISG group showed significant change ($p < 0.01$) in the studied iron status marker {(Ferritin: F (2,50) = 31.29; p = 0.000)} and inflammatory markers {(HsCRP: (F (2,50) = 10.91; p = 0.000); (cortisol: F (2,50) = 3.80; 0.029)} when analyzed in longitudinal manner, whereas iron and sTfR changed

Table 2 Daily versus weekly IFA tablets supplementation affecting haematological parameters, birth weight, gestation length and mode of delivery.

Parameters	Non anaemic pregnant women			Anaemic pregnant women	
		DISG	WISG	DISG	WISG
Haemoglobin (g/dl)	Baseline	12.11 ± 0.12	12.06 ± 0.12	10.12 ± 0.14	9.92 ± 0.12
	Second visit	11.46 ± 0.14	11.28 ± 0.17	10.88 ± 0.24	9.72 ± 0.25
	Pre delivery	11.84 ± 0.17	11.56 ± 0.20	11.08** ± 0.26	9.91 ± 0.28
	Postpartum	12.33* ± 0.22	11.65 ± 0.23	11.62 ± 0.23	11.57 ± 0.44
Haematocrit (%)	Baseline	35.52 ± 0.37	35.48 ± 0.37	30.96 ± 0.54	31.04 ± 0.58
	Second visit	33.46 ± 0.38	33.32 ± 0.46	31.33 ± 0.76	28.50 ± 1.20
	Postpartum	35.23 ± 1.39	35.33 ± 0.67	34.92 ± 0.60	33.17 ± 1.90
RBC count ($\times 10^6$/μl)	Baseline	4.09 ± 0.05	4.11 ± 0.07	3.67 ± 0.12	3.61 ± 0.12
	Second visit	3.71 ± 0.05	3.71 ± 0.04	3.70 ± 0.10	3.35 ± 0.14
	Postpartum	4.16 ± 0.08	4.04 ± 0.12	4.05 ± 0.12	3.97 ± 0.25
MCV (fl)	Baseline	86.92 ± 0.88	86.83 ± 1.40	86.67 ± 3.49	88.00 ± 2.71
	Second visit	90.46 ± 0.90	89.94 ± 1.22	84.95 ± 2.88	85.45 ± 2.71
	Postpartum	84.56 ± 3.06	88.37 ± 1.88	86.76 ± 2.32	84.33 ± 2.40
MCH (pg/cell)	Baseline	29.64 ± 0.31	29.56 ± 0.55	28.55 ± 1.32	28.30 ± 0.99
	Second visit	31.17 ± 0.41	30.54 ± 0.44	28.73 ± 1.07	29.04 ± 1.01
	Postpartum	29.70 ± 0.52	29.37 ± 0.62	28.90 ± 0.92	28.76 ± 1.05
Gestation length (days)		270.6 ± 1.97	269.5 ± 2.04	271.3 ± 2.85	270.5 ± 2.64
Vaginal delivery (%)		71.5	52	71.5	69.3
Caesarean delivery (%)		28.5	48	28.5	30.7
Birth weight (gm)		2825 ± 89.71	2841 ± 92.26	2819 ± 139.8	2918 ± 86.78

DISG – daily iron folic acid supplementation group; WISG – weekly iron folic acid supplementation group; RBC count – red blood corpuscle count; MCV – mean corpuscular volume; MCH – Mean corpuscular haemoglobin.
Data expressed as mean ± SE; Significance $^{*}P < 0.05$, $^{**}P < 0.01$.

non-significantly throughout the studied intervals. Furthermore, the postpartum period referred to as the period of lowest iron deficiency risk (National Research Council, 1990) as the expanded red cell mass contracts at delivery resulting in the increased body iron stores (Bothwell, 1995) and continuing the IFA tablets daily might contribute to the excessive increased serum iron levels and reserves of the body that was found in the current study.

Though, weekly IFA supplementation resulted in anaemia at postpartum (anaemia defined at postpartum; Hb < 12.3 g/dl (Bentley, 1985)) without iron deficiency (iron deficiency defined at postpartum; serum ferritin < 15 ng/ml (Milman et al., 2000)) and significant decrease in serum level of iron at second visit; still the decline was well above the cut-off levels defined for the deficiency of respective markers and also there were no marked differences obtained between DISG and WISG in rest of the studied parameters. All the studied parameters viz. iron (F (2, 20) = 3.71; p = 0.043), HsCRP (F (2, 52) = 14.95; p = 0.000) and cortisol (F (2, 52) = 16.18; p = 0.000) except sTfR and ferritin were significantly different in WISG when analyzed by repeated ANOVA. Furthermore, the pre-delivery Hb was comparable in both the supplementation groups stating weekly supplementation is as effective as daily supplementation in improving Hb level till term delivery. The body iron reserves in terms of sTfR, ferritin and Hb were well maintained up to the initiation of third trimester in both the supplemental schedules (Table 3).

Thus, it can be summarized that weekly IFA supplementation could be a better approach in terms of cost effectiveness and producing comparable results in non-anaemic pregnant women. It was noted that it may contribute to the incidence of mild to moderate side effects and slight increase chances of caesarean delivery but the results may be confounding due to multiple factors.

3.2. Daily versus weekly IFA supplementation in anaemic pregnant women

3.2.1. Amount and regularity of IFA tablets intake from baseline to second visit

The mean of IFA tablets consumed was 89.4 in DISG with 77.7% women being highly regular and 22.2% irregular as compared to 24.2 in WISG where 100% were found highly regular. Though the symptoms that appeared due to IFA intake were more in WISG it did not affect the compliance rate, which might be due to the less severity of the observed symptoms. However, the percentage of women who withheld IFA tablets intake due to forgetfulness was 22.2% in DISG whereas this percentage found to be nil in WISG (Table 1).

3.2.2. Amount and regularity of IFA tablets intake from second visit to 6 weeks postpartum

During this period, the mean IFA tablet intake was 116.3 in DISG with regularity of intake being 66.6% highly regular, 22.2% irregular and 11.1% highly irregular as compared to 31.6 in WISG where 62.5% were found highly regular, 25% irregular and 12.5% highly irregular. In DISG, 11.1% women withheld IFA due to side effects and 55.5% withheld due to forgetfulness compared to 12.5% and 37.5% in WISG

Table 3 Daily versus weekly iron supplementation affecting the iron status markers.

Parameters	Non-anaemic pregnant women		Anaemic pregnant women	
	DISG	WISG	DISG	WISG
Haemoglobin (g/dl)	Baseline	12.11 ± 0.12	12.06 ± 0.12	10.12 ± 0.14	9.92 ± 0.12
	Second visit	11.46 ± 0.14	11.28 ± 0.17	10.88 ± 0.24	9.72 ± 0.25
	Pre delivery	11.84 ± 0.17	11.56 ± 0.20	11.08 ± 0.26**	9.91 ± 0.28
	Postpartum	12.33 ± 0.22*	11.65 ± 0.23	11.62 ± 0.23	11.57 ± 0.44
S. Ferritin (ng/ml)	Baseline	34.52 ± 5.12	26.69 ± 4.12	35.39 ± 7.18	34.77 ± 4.59
	Second visit	53.08 ± 6.78	33.51 ± 5.49	51.99 ± 9.91	34.38 ± 7.63
	Postpartum	132.3 ± 21.33**	37.04 ± 9.16	135.7 ± 25.74*	52.24 ± 12.17
S. Iron (μg/dl)	Baseline	115.5 ± 6.57	110.7 ± 6.96	97.99 ± 5.79	91.04 ± 4.88
	Second visit	133.7 ± 8.42**	102.7 ± 7.62	119.5 ± 7.63***	73.29 ± 6.26
	Postpartum	132.7 ± 14.48*	97.2 ± 6.76	96.9 ± 6.29	96.80 ± 11.10
S. sTfR (μg/ml)	Baseline	1.87 ± 0.28	1.78 ± 0.23	1.49 ± 0.33	1.41 ± 0.25
	Second visit	1.46 ± 0.13	2.16 ± 0.39	1.73 ± 0.29	1.95 ± 0.29
	Postpartum	1.36 ± 0.15	1.44 ± 0.35	1.69 ± 0.36	1.67 ± 0.31
S. EPO (mIU/ml)	Baseline	18.40 ± 1.74	20.45 ± 10.20	15.77 ± 2.70	14.17 ± 3.83
	Second visit	27.41 ± 5.47	30.33 ± 6.63	31.94 ± 6.21	68.67 ± 15.61$^{\alpha}$
	Postpartum	13.33 ± 3.5	15.00 ± 2.91	12.00 ± 1.67	13.00 ± 1.66
S. HsCRP (mg/l)	Baseline	4.84 ± 0.88	4.33 ± 1.03	5.53 ± 1.05	4.16 ± 0.65
	Second visit	7.60 ± 1.30	6.26 ± 1.28	3.31 ± 0.76	4.28 ± 1.32
	Postpartum	1.65 ± 0.60	2.33 ± 0.56	8.40 ± 1.39$^{\beta}$	5.05 ± 1.13
S. Cortisol (ng/ml)	Baseline	98.09 ± 20.37	97.13 ± 12.88	134.0 ± 11.76	112.8 ± 11.02
	Second visit	155.5 ± 20.90	155.6 ± 12.19	202.7 ± 13.32	185.9 ± 20.57
	Postpartum	87.06 ± 12.73	98.05 ± 12.63	106.1 ± 15.19	79.68 ± 14.48

DISG – daily iron folic acid supplementation group; WISG – weekly iron folic acid supplementation group; S. sTfR – serum soluble transferring receptor; S. EPO – serum erythropoietin; S. HsCRP – serum high sensitivity C-reactive protein.
Data expressed as mean ± SE; Significance $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$, $^{\alpha}P \leqslant 0.06$, $^{\beta}P \leqslant 0.09$.

respectively. The symptoms like nausea and vomiting were observed more in WISG as compared to DISG (Table 1).

3.2.3. Haematological markers and delivery outcomes

There was no significant difference observed in most of the studied haematological parameters, birth weight, gestation length and mode of delivery (Table 2). Nevertheless, pre-delivery haemoglobin was found to be significantly high in DISG compared to WISG ($p < 0.01$) achieving the value near non-anaemic haemoglobin level at term. In addition, after analyzing the variables using repeated measure ANOVA, a significant change was observed in Hb (F (2, 38) = 25.17; p = 0.000); Hct (F (2, 38) = 21.10; p = 0.000) and RBC count (F (2, 38) = 3.63; p = 0.036) in DISG and Hb (F (2, 44) = 11.18; p = 0.000); Hct (F (2, 44) = 12.90; p = 0.000) and RBC count (F (2, 42) = 5.84; p = 0.006) in WISG. The results revealed that the significant change in haematological parameters are comparable in both the groups.

3.2.4. Iron status markers

In anaemic pregnant women, both the supplementation regimen failed to achieve non-anaemic Hb level at studied time interval except pre-delivery Hb where it was significantly high in DISG. However, it is noteworthy that most of the studied parameters were found comparable. The significant increase at the postpartum serum level of ferritin ($p < 0.05$) was observed in DISG, which could be explained, by non-significant increased level of inflammatory protein HsCRP ($p \leqslant 0.09$) (Table 3). Since serum ferritin being an indicator of iron stores found to be elevated in inflammatory conditions that limit its utility as a sole marker for defining iron deficiency. Thus, it should be used along with other more sensitive iron markers like sTfR whose level remained quite stable and rose only in the condition of insufficient iron supply to the erythrocytes due to exhausted iron reserves (Baynes, 1994). Furthermore, the significant difference between DISG and WISG was also observed in serum iron level at second visit ($p < 0.0001$) but again this could be the result of enhanced erythropoiesis under the influence of erythropoietin hormone whose level was found non-significantly high in WISG compared to DISG ($p \leqslant 0.06$) (Table 3). In various studies an inverse relation was observed between Hb and iron status with erythropoietin hormone (Barton et al., 1994; Cook, 1985; McMullin et al., 2003) that is similar to the current findings. The enhanced erythropoiesis in WISG at second visit could be explained by the activation of homoeostatic mechanism of the body in response of low level of Hb, which in turn leads to increased iron requirement resulting in iron depletion. However, it has been reported that the iron supplementation imposed a check on this erythropoietic drive of the body (Barton et al., 1994; McMullin et al., 2003). Thus it may be inferred that weekly IFA supplementation could not provide sufficient iron needed for erythropoiesis resulting in the depletion of serum iron level at second visit. While analyzing the data using repeated measure statistical test, most of the parameters viz. ferritin (F (2, 50) = 31.29; p = 0.000); sTfR (F (2, 54) = 17.38; p = 0.000) and cortisol (F (2, 54) = 30.49; p = 0.000) were significantly changed from baseline to 6 weeks

postpartum and ferritin ($F(2,54) = 26.62$; $p = 0.000$); HsCRP ($F(2,54) = 3.60$; $p = 0.034$); cortisol ($F(2,54) = 5.55$; $p = 0.006$); Iron ($F(2,8) = 7.20$; $p = 0.016$) in WISG. This shows that most of the iron and inflammatory markers changed during the course of pregnancy. Mainly, weekly supplementation caused decline in iron markers and increase in inflammatory markers.

The results of the study warrant using weekly iron supplementation during anaemic pregnancy as anaemia itself contributes to enhanced erythropoiesis that results in the increased iron requirements leading to iron deficiency and further iron exhaustion. Daily iron supplementation was found to be comparatively safer approach as the Hb level was maintained near the cut-off value to define anaemia during pregnancy.

4. Conclusion

The concluding remarks include that daily rather than weekly iron supplementation is a more effective way to combat anaemia during pregnancy in anaemic pregnant women. However, in non-anaemic pregnant women weekly iron supplementation is as effective as daily dose in terms of birth weight, gestation length and cost effectiveness but at a price of slightly more side effects and increased chances of caesarean deliveries.

Trial registration

Clinical Trial Registry-India: CTRI/2014/10/005135 [Registered on: 22/10/2014].

Conflict of interest

Authors declared no conflict of interests.

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