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Impact of daily and weekly iron supplementation to women in pregnancy and puerperium on haemoglobin and iron status six weeks postpartum: results from a community-based study in Bangladesh

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Abstract

***Background*:** Anaemia and iron-deficiency anaemia in women are global problems that are prevalent throughout the reproductive cycle. Data are scarce on whether iron supplementation in pregnancy and puerperium has a sustained effect on haemoglobin concentration.

Objective: To assess whether there is a dose effect of iron supplementation in pregnancy and puerperium on haemoglobin 6 weeks after delivery, and compare the effectiveness of daily and weekly dose regimens at 6 weeks postpartum.

Design: 50 antenatal centres were assigned randomly to 1×60 mg iron daily or 2×60 mg once weekly. Data are reported for 146 women (daily, $n = 67$; weekly, $n = 79$): haemoglobin, serum ferritin (sFt) and serum transferrin receptors (sTfR) at baseline and at 6 weeks postpartum. Tablet intake was monitored using pill-bottles equipped with electronic counting devices.

Results: There was a dose effect of iron supplementation on haemoglobin concentration at 6 weeks postpartum. Endpoint attained haemoglobin, sFt and sTfR did not differ between daily and weekly groups, although a larger increment of sFt was found in the daily group ($p = 0.03$).

Conclusions: Effects of iron supplementation in pregnancy and puerperium were observed at 6 weeks after delivery. The size of the effect was dependent on the number of tablets, not on daily or weekly regimen. It is not known whether the effects of iron supplementation in pregnancy are sustained into the next pregnancy.

Keywords: *Bangladesh*, *haemoglobin*, *iron status*, *iron supplementation*, *pregnancy*, *puerperium*.

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Introduction

Although anaemia has the highest prevalence during pregnancy, it is common throughout the reproductive cycle (1–3). The knowledge base is weak regarding whether repeated and short-spaced pregnancies deplete iron stores and further increase the prevalence of anaemia in the female population (4, 5). Little is known about the effect of iron supplementation in pregnancy on postpartum iron and haemoglobin status, although some studies have claimed that such interventions reduce the overall prevalence of anaemia in women of reproductive age (6, 7).

The current recommendation is to provide supplements of 60 mg iron and 400 μg folic acid daily during pregnancy and puerperium (8). In general,

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however, iron supplementation programmes have met with limited success (9). To increase the programme effectiveness, an intermittent dose frequency of iron supplementation has been suggested (10), resulting in a number of field trials to test the efficacy and effectiveness of weekly regimens. These trials compared the effect of iron supplementation in daily versus weekly regimens in different population groups, including pregnant women (11–13). Most of these trials reported contradictory findings as to whether one regimen was equivalent to or better than the other. A meta-analysis comparing the two dose frequencies concluded that although a daily regimen was better than a weekly one, both were efficacious in preventing a fall in haemoglobin concentration during pregnancy (14). None of the studies compared the effect of daily and weekly prenatal regimens of iron supplementation on outcomes in the postpartum period.

The postpartum haematological status is dependent not only on haemoglobin concentration and iron status in pregnancy, but also on the amount of bleeding during delivery and the use of iron supplements after childbirth. A recent community-based trial compared the effect of iron supplementation provided in daily and weekly frequencies (15). The study indicated that a 12 week daily regimen during pregnancy had a greater effect on prenatal haemoglobin concentration than a weekly regimen, but also suggested that after a longer supplementation period the effect of a weekly regimen could approach that of a daily one. The aims of this study are to investigate whether iron supplementation during pregnancy and puerperium has a dose effect on haemoglobin 6 weeks after delivery, and whether there is any differential effect between daily and weekly regimens at 6 weeks postpartum.

Subjects and methods

Study area

The study took place in Mymensingh district in northern Bangladesh. The area is representative of the plain land in the country, with a high population density, high rates of illiteracy and malnutrition, and limited access to health services. There were no reported cases of malaria in the study population and the hookworm prevalence was low, around 1% (16). To assist in the implementation of the government health policies, BRAC, the largest national non-governmental organization in Bangladesh, distributes iron tablets to pregnant women through its community-based antenatal care centres. Each centre covers about 1000 people and is managed by a female community health worker.

Iron folate supplementation

Fifty antenatal care centres were randomly assigned to prescribe either daily (routine programme) or weekly dosages of iron supplementation. Women who were assigned to the daily supplementation group were advised to take one iron tablet every evening, and women in the weekly group to take two iron tablets every Friday, one in the morning and the other in the evening. Each tablet contained 60 mg elemental iron as ferrous sulfate and 250 μg folic acid. The supplements were red, film coated and produced in Norway. The women were advised to take iron tablets from the day of recruitment during pregnancy and continue until 6 weeks postpartum.

Subjects

Through household visits, all women of reproductive age served by 50 antenatal care centres were screened for pregnancy. Information on socioeconomic status (SES) and reproductive history was collected from identified pregnant women with a fundal height < 22 cm that was judged to correspond to gestational age < 24 weeks (17), and they were encouraged to receive services from their respective centre. The first four or five women who appeared at the respective antenatal care centre on a fixed day and fulfilled the inclusion criteria were included in the study. The inclusion criteria were fundal height 14–22 cm, yet to start taking iron supplements during the current pregnancy, and haemoglobin concentration ≥ 80 g l^{-1}. In total, 209 pregnant women were enrolled in the iron supplementation trial, 104 in the daily and 105 in the weekly group. This sample size in each group was determined to be able to demonstrate a difference in haemoglobin of 5 g l^{-1} between weekly and daily dose regimens, evaluated after 12 weeks of supplementation during pregnancy with a given power of 0.80 and a significance level of 0.05, plus an allocation for dropout of 20%. Informed consent was obtained from each recruited woman at enrolment.

Methods

Data on SES, reproductive history, fundal height, intake of iron tablets and haemoglobin status were collected by trained field workers as reported in detail elsewhere (15). An SES score was con-

structed that ranged from 3 (highest SES) to 0 (lowest SES). The symphysis–fundal height was measured to the nearest 0.1 cm along the longitudinal uterine axis, the woman having emptied her bladder and lying in a supine position with the legs extended. Female field workers performed the measurement after training at an obstetric unit and having achieved satisfactory results of standardization of measurements. Intake of iron tablets was monitored by the Medication Event Monitoring System (MEMS®), a specialized pill bottle with an electronic counting device embedded in the cap (18). Each time the bottle was opened and closed the date and time of the event was recorded, which was considered as one tablet taken. For a 12 week supplementation period a total of 100 tablets in the daily and 30 tablets in the weekly groups were delivered. At the end of the 12 week period, the MEMS were returned and replaced with an ordinary cap with sufficient iron tablets to last until the end of 6 weeks postpartum.

In the analysis of this paper, tablet intake during the first 4 weeks was used as an indicator of the total number of tablets taken. Information from MEMS monitoring of tablet intake over 12 weeks was available for 132/146 women. The correlation coefficient between the first 4 weeks and the 12 week period was 0.89. Thus, in order not to reduce further the size of the data available for analysis, data on the first 4 weeks were used.

Data on side-effects to iron supplementation were collected at the end of the first month of supplementation by probing seven of the most relevant symptoms. Side-effects did not have any significant influence on compliance in either the daily or weekly group; details have been reported elsewhere (19).

Venous blood was collected from non-fasting, seated participants in an untreated evacuated tube at baseline and at 6 weeks postpartum during the centre visit from 09:00 to 11:00 h. Haemoglobin concentration was assessed on venous blood using the HemoCue® system. Anaemia was defined according to the World Health Organization (WHO) as haemoglobin concentration < 110 g l^{-1} in pregnant women and < 120 g l^{-1} in postpartum women (1). Tubes containing the blood samples were kept on a test-tube stand for 1 h allowing for clot formation and, thereafter, the samples were transported on ice within 4 h to the field laboratory. No occurrence of haemolysis was reported. The blood samples were centrifuged at room temperature for 5 min, and the serum was taken off and frozen at -20°C. The frozen samples were transported on dry ice to the ICDDR,B laboratory in Dhaka and stored at minus 70°C until analysis. At the end of the study, the samples were transported on dry ice for analysis at the Department of Nutrition, University of California Davis, USA. Serum ferritin (sFt) was assessed by radioimmunoassay (RIA; Diagnostic Products, San Diego, CA, USA). sFt values < 12 μg l^{-1} were considered low, reflecting depleted iron stores (20). Serum transferrin receptors (sTfR) were assessed by an enzyme-linked immunosorbent assay (ELISA; Ramco Laboratories, Houston, TX, USA). sTfR values > 8.5 mg l^{-1} were considered high, indicating tissue iron deficiency (21). For the purpose of this study iron deficiency has been defined as having low sFt and/or high sTfR.

The ethics committee of the Bangladesh Medical Research Council (BMRC), Bangladesh, and the Research Ethics Committee of the Medical Faculty, Umeå University, Sweden, approved the study protocol.

Data analysis

The distributions of sFt and sTfR were skewed and, therefore, normalized by a natural logarithmic transformation before analysis. Medians were used as a measure of their central tendency, while means were used for haemoglobin concentration and other variables with a normal distribution. Original values were used for haemoglobin concentration and transformed values for sFt and sTfR to perform all statistical tests. Differences in baseline characteristics between groups were tested by Student's t-test. The differences in haemoglobin concentration, sFt and sTfR between daily and weekly supplementation groups were tested by Student's t-test. The number of iron tablets taken during the first 4 weeks of supplementation was arbitrarily divided into three equally sized parts, representing low, medium and higher intake. These levels were used when testing the effect of iron supplementation during pregnancy on haemoglobin concentration at 6 weeks postpartum in an analysis of variance (ANOVA) that was adjusted for initial haemoglobin concentration. Bonferroni's test was applied, contrasting haemoglobin concentration for low and higher intake levels. The difference between categorical variables was tested by the χ^2-test. Statistical significance was defined as $p < 0.05$. Data were analysed using SPSS for Windows, Release 7.5.1 (SPSS, Chicago, IL, USA).

Results

Of the 209 pregnant women who were enrolled in the iron-supplementation trial, 63 were lost to follow-up before 6 weeks postpartum. The reasons included errors in MEMS® that resulted in loss of iron tablet intake data ($n = 29$), refusals at the final blood sampling ($n = 31$) or withdrawal from the trial during pregnancy ($n = 3$). Data are reported on 146 women who had complete information on intake of iron tablets during weeks 1–4 and haemoglobin concentration, sFt and sTfR at baseline and at 6 weeks postpartum (daily, $n = 67$; weekly, $n = 79$) (Table 1).

Comparison of baseline characteristics between women who were included in the analyses and women lost to follow-up showed that, in the daily group, more women with lower fundal height were lost, and in the weekly group, more women with lower haemoglobin concentration were lost (Table 2). Among women included in the analyses there was no difference in the baseline characteristics, except that sFt was higher in the weekly group ($p = 0.06$).

To evaluate the trial effectiveness of iron supplementation after childbirth, the change in and attained values of haemoglobin concentration, sFt and sTfR at 6 weeks postpartum were compared between daily and weekly supplementation regimens. Haemoglobin concentration and sTfR did not significantly differ between the groups in terms of either total increment over the period or the attained level at 6 weeks postpartum (Table 3). However, a larger increment of sFt was observed among women in the daily regimen. Furthermore, there was no significant difference between daily and weekly regimens in terms of the prevalence of anaemia (27 vs 22%), low sFt (8 vs 9%) or high sTfR (24 vs 16%), respectively, at 6 weeks postpartum.

Women with a lower initial haemoglobin concentration (< 115 g l^{-1}) were selected to evaluate trial effectiveness in a group of women with a

Table 1. Number of women enrolled in the study and reasons for loss to follow-up

		Iron-supplementation regimen	
	n	Daily	Weekly
Enrolled in the trial	209	104	105
No compliance data from week 1–4 owing to errors in MEMS®	29	15	14
Withdrew from the trial	3	1	2
Refused blood sampling at 6 weeks postpartum	31	21*	10
Had complete information at 6 weeks postpartum	146	67	79
Baseline Hb < 115 g l^{-1}	93	46	47

Hb: haemoglobin concentration.

* Significant difference between daily and weekly ($p < 0.05$).

Table 2. Baseline characteristics of the women enrolled in the study and those lost to follow-up

	With complete information			**Lost to follow-up**		
	Daily ($n = 67$)	Weekly ($n = 79$)	All ($n = 146$)	Daily ($n = 37$)	Weekly ($n = 26$)	All ($n = 63$)
Age (years)	24.8 ± 6.0	24.0 ± 5.7	24.3 ± 5.9	23.7 ± 6.2	23.5 ± 6.2	23.6 ± 6.2
Parity (*n*)	1.5 ± 1.3	1.4 ± 1.3	1.5 ± 1.2	1.4 ± 1.2	1.4 ± 1.3	1.4 ± 1.2
Fundal height (cm)	17.3 ± 2.3^{A}	17.2 ± 2.0	17.2 ± 2.2	16.2 ± 1.3^{A}	17.4 ± 2.6	16.7 ± 2.0
SES score	1.3 ± 0.6	1.1 ± 0.8	1.2 ± 0.7	1.2 ± 0.8	1.2 ± 0.8	1.2 ± 0.8
Hb (g l^{-1})	110 ± 15	112 ± 13^{B}	111 ± 14	111 ± 13	103 ± 13^{B}	107.8 ± 13.6
sFt (μg l^{-1})[a]	12.4^{C}	20.3^{C}	14.7	13.4	13.7	13.4
sTfR (mg l^{-1})[a]	6.2	5.9	6.0	6.3	6.3	6.3

Data are shown as means $\pm$ SD or [a] medians.

SES: socioeconomic status; Hb: haemoglobin concentration; sFt: serum ferritin; sTfR: serum transferrin receptor.

Corresponding superscript letters indicate significant differences: ^{A,B} $p < 0.05$, ^{C} $p = 0.06$.

Table 3. Haemoglobin concentration (Hb) and iron status at baseline and 6 weeks postpartum (PP) by dose frequency of iron supplementation; full sample and a subset with initial Hb < 115 g l^{-1}

	Iron-supplementation frequency					
	Full sample			Subset (Hb < 115 g l^{-1})		
	Daily ($n = 67$)	Weekly ($n = 79$)	*p*	Daily ($n = 46$)	Weekly ($n = 43$)	*p*
Hb (g l^{-1})						
At baseline	110 ± 15	112 ± 13	0.34	102 ± 9	104 ± 8	0.39
Change 6 weeks PP	18 ± 12	18 ± 18	0.90	26 ± 17	22 ± 17	0.32
Attained 6 weeks PP	128 ± 18	130 ± 18	0.55	128 ± 17	126 ± 16	0.58
SFt (μg l^{-1})[a]						
At baseline	12.4	20.3	0.06	8.8	19.0	0.04
Change 6 weeks PP	41.7	32.0	0.04	47.5	28.2	0.002
Attained 6 weeks PP	57.6	57.3	0.59	58.0	48.7	0.24
sTfR (mg l^{-1})[a]						
At baseline	6.2	5.9	0.12	7.2	6.3	0.02
Change 6 weeks PP	−0.5	−0.1	0.68	−0.8	−0.1	0.14
Attained 6 weeks PP	4.9	4.9	0.58	5.7	5.7	1.0

Data are shown as means ± SD or [a] medians.
sFt: serum ferritin; sTfR: serum transferrin receptor.

potentially higher response to supplementation. In this subset of women, similarly to the results of the full set, haemoglobin concentration and sTfR did not significantly differ between the groups (Table 3). However, a larger increment of sFt was observed in the daily regimen than in the weekly at 6 weeks postpartum. A significantly ($p < 0.01$) larger sFt increment in the daily regimen was also confirmed in a multiple linear regression analysis controlling for the initial sFt (data not shown).

Using the same subset of women with initial haemoglobin < 115 g l^{-1}, the effect of tablet intake on haemoglobin concentration at 6 weeks postpartum was tested by ANOVA, controlling for initial haemoglobin concentration. Low, medium and high tablet intake groups had mean haemoglobin concentrations at 6 weeks postpartum of 121, 127 and 132 g l^{-1}, respectively (contrasting lowest and highest intake group, $p = 0.046$). The corresponding proportions of anaemia were 42, 21 and 10%, respectively (test for trend $p = 0.004$).

Discussion

The results showed that the effect of prenatal and puerperial iron supplementation was sustained at 6 weeks after delivery. Further, trial effectiveness of iron supplementation during pregnancy and puerperium did not differ between women assigned to the daily and the weekly regimen, evaluated as haemoglobin concentration at 6 weeks postpartum. However, the daily regimen was more effective in increasing and sustaining iron status after childbirth.

More women in the daily supplementation group were lost to follow-up because they refused blood sampling at 6 weeks postpartum (Table 1). While these women had a lower fundal height than women with complete information in the daily group, their initial haemoglobin concentration was similar. In the weekly group, more women with lower haemoglobin concentrations were lost to follow-up. While these losses did not result in a difference in baseline characteristics between daily and weekly regimens, there was a difference in sFt between the groups. The lower initial sFt in the daily regimen may have led to an underestimation of sFt at 6 weeks postpartum in this group. However, it may have overestimated the increment as women with lower sFt can be expected to have a larger response.

A major focus of this study was to compare the trial effectiveness of iron supplementation between women assigned to two frequencies of iron supplementation. Interventions, including the daily and weekly regimens, were randomized and, therefore, the potential confounding factors were expected to be randomly allocated between groups. The initial sFt was the only baseline characteristic that was not equally distributed between groups. This may

have biased the impact on final sFt when compared between groups. However, the baseline difference was adjusted for by including the initial sFt in a linear regression model while comparing the impact at 6 weeks postpartum. The comparison of other baseline characteristics revealed no significant difference between the daily and weekly supplementation groups. Thus, it can be concluded that none of these characteristics, including SES, confounded the comparison between groups.

Daily versus weekly regimens

At 6 weeks postpartum, haemoglobin concentration significantly increased and corresponding anaemia decreased over time, but did not differ between daily and weekly supplementation groups either in full sample or in the subset of women with lower haemoglobin concentration (< 115 g l^{-1}). Several possibilities may be discussed to explain the finding of no difference in the effect at 6 weeks postpartum.

One possibility is that neither women in the daily nor those in the weekly group responded to iron supplementation. The lack of response could be attributable to reasons including limited compliance or the presence of other factors that limit the response, such as vitamin A deficiency (3, 22). The observed increase in haemoglobin concentration could, in such case, be due to a reversal of haemodilution that occurs in late pregnancy (23) or positive iron balance after childbirth (24). However, in the present study, this possibility was ruled out by showing that number of iron tablets was predictive of haemoglobin concentration at 6 weeks postpartum and, thus, a lack of response would not explain the lack of difference in effect between the daily and weekly dosages of iron supplementation. This is also supported by the larger sFt response in the daily group.

The next possibility is that the total numbers of tablets consumed in the two supplementation regimens were similar and, therefore, a similar effect was produced. Information on tablet intake was not collected over the full supplementation period and it is, thus, not possible to confirm or reject this possibility. However, for the first 4 weeks of supplementation, it has been shown that there is a considerable overlap in tablet intake (15), as many of the women in the daily group took fewer tablets and women on the weekly regimen took more tablets than prescribed. It is, thus, conceivable that differential compliance between the groups led to a similar number of tablets consumed at 6 weeks postpartum.

The third possibility is that the number of tablets provided by weekly regimen was sufficient to produce maximum response. This was the explanation for the smaller than expected difference in response between daily and weekly supplementation evaluated after 3 months of supplementation during pregnancy (15), but it is not possible to conclude whether this was the case at 6 weeks postpartum. The mean haemoglobin concentration for healthy, well-nourished, non-pregnant women has been reported to be 135 g l^{-1} (25), suggesting that neither women on the daily nor those on the weekly regimen fully reached a normal haemoglobin concentration. Still, they may have reached the maximally achievable effect by iron supplementation in this setting.

Thus, the most likely explanation for a lack of difference in haemoglobin response at 6 weeks postpartum between daily and weekly iron supplementation is overlapping distributions by the two regimens. It should be noted that neither regimen fully normalized the postpartum haemoglobin concentration; this probably reflects the multiple causes of anaemia in pregnancy and puerperium.

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