Anaemia and iron deficiency in women

Impact of iron supplementation during pregnancy in rural Bangladesh

S.M. Ziauddin Hyder
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Umeå 2002
Dedicated

to all mothers in Mymensingh Sadar upazila, Bangladesh, who participated in the study
Abstract

Iron deficiency anaemia is a global public health problem that affects women in all stages of the reproductive cycle. Current iron supplementation strategies in public health settings have met with limited success. Alternatives with intermittent dose frequency have been suggested to reduce side effects, increase compliance and iron absorption and, thereby, improving effectiveness. The objectives of this thesis were to assess the anaemia problem in rural Bangladeshi women, and to compare the impact of a daily and a weekly iron supplementation on haemoglobin (Hb) outcome in pregnancy and puerperium.

In a survey of 179 rural non-pregnant women (15-45 years), anaemia (Hb <120 g/L) was highly prevalent in all social strata, however, severe anaemia (Hb <70 g/L) was uncommon.

An iron supplementation trial was conducted in 50 antenatal care centres randomly assigned to provide women with either 60 mg iron daily or 2 x 60 mg once weekly from second trimester to 6 weeks post partum. Compliance was monitored over 11 weeks of supplementation during pregnancy by use of a pill-bottle equipped with an electronic counting device. Side effects were assessed by recall after 4 weeks. Hb was assessed at baseline, after 4, 8 and 12 weeks and at 6 weeks after delivery. Iron status was assessed at baseline and at 6 weeks post partum by serum ferritin (sFt) and serum transferrin receptors (sTfR).

Half of the pregnant women (n = 214) at baseline had anaemia (Hb <110 g/L) while none had severe anaemia. Four out of five cases of moderate anaemia (Hb 70-99 g/L) and every second case of mild anaemia (Hb 100-109 g/L) had indications of iron deficiency (sFt <12 µg/L and/or sTfR >8.5 mg/L). When evaluating the effect of iron supplementation on haemoglobin concentration no difference was found per iron tablet taken as daily or weekly dose schedule. The first 20 tablets consumed produced most of the effect, and the maximum response was achieved after about 40 tablets. Side effects were more common in the weekly group, but had limited influence on compliance. Compliance was higher among those taking tablets on a weekly basis. A significantly larger haemoglobin response was achieved by a 12-week daily regimen as compared to weekly. At 6 weeks post partum, there was a dose-dependent effect of iron supplementation that did not differ between the two regimens.

Reduction of anaemia is dependent on the actual number of iron tablets consumed rather than on supplementation regimen. The current international recommendation of iron supplementation in pregnancy seems to be unnecessarily high. Since most of the improvement in haemoglobin concentration was found to occur early in treatment, the initial supplementation dose in pregnancy should be set as high as possible, which may then be followed by a lower (intermittent) dose. The study provides evidence that the impact of iron supplementation in pregnancy could be sustained after childbirth.

Keywords: Anaemia, iron deficiency, iron supplementation, non-pregnant, pregnant, post partum, efficacy, trial effectiveness, weekly dose frequency, Bangladesh.
Abstract in Bangla

†jŠn NvUwZ RwbZ i³¯^íZv wek¦e¨vcx GK Rb¯^v¯'¨ mgm¨v, hv gwnjv‡`i cÖRbb P‡µi cÖwZwU ¯—‡iB ¯^v¯'¨nvbxi SzuwK evovq| G mgm¨v mgvav‡bi Rb¨ Ghver cÖPwjZ ˆ`wbK †jŠn ewo †meb Lye GKUv Kvh©Ki nqwb| G Ae¯'vi cwi‡cÖw¶‡Z weKí e¨e¯'v wn‡gv‡M−vweb 120 MÖvg/wjUv‡ii bx‡P) AwZ D"P cÖ‡Kvc tLvMHO (73%)) Mf©eZx bq Ggb 15-45 eQi eqmx 179 Rb my¯' gwnjvi g‡a¨ GK Rwi‡c i³¯^íZvi (wn‡gv‡M−vweb 70 MÖvg/wjUv‡ii wb‡P) AwZ D"P cÖ‡Kvc tLvMHO (73%)) Mf©eZx B Gi cwbvbæ`©v Z n‡jI gwKí³ "‡Zv AwqiÉ A l‡' i³ jšv en Kvh©Ki n‡q‡Q| eZ©gvb M‡elYv cÖKíwUi j¶¨ n‡jI evsjv‡`‡k MÖvgxY gwnjv‡`i gv‡S i³¯^íZvi cÖ‡Kvc †`Lv Ges Mf©eZx qu mbRb‡bi ci guq‡i³³ "‡Zv Kgbvwbæ`©v u³ évi "wK Ges mš—vbK weiz‡Z jšv en tmebi Kvh©KiZv hPvB KiJ

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Original papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals*.


II. Hyder SMZ, Persson LÅ, Chowdhury AMR, Lönnerdal B, Ekström E-C. Anaemia and iron deficiency during pregnancy in rural Bangladesh. Submitted.


IV. Hyder SMZ, Persson LÅ, Chowdhury AMR, Ekström E-C. Do side effects reduce compliance to iron supplementation? A study of daily and weekly dose regimens in pregnancy. Journal of Health, Population and Nutrition. Accepted.

V. Hyder SMZ, Persson LÅ, Chowdhury AMR, Lönnerdal B, Ekström E-C. Impact of daily and weekly iron supplementation to women in pregnancy and puerperium on haemoglobin and iron status at 6 weeks post partum: results from a community-based study in Bangladesh. Manuscript.

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Glossary and definitions

**Anaemia** is defined as haemoglobin concentration in non-pregnant women less than 120 g/L and in pregnant women less than 110 g/L (1).

**Mild anaemia** is defined as haemoglobin concentration in non-pregnant women from 100 to 119 g/L and in pregnant women from 100 to 109 g/L (1).

**Moderate anaemia** is defined as haemoglobin concentration both in non-pregnant and pregnant women from 70 to 99 g/L (1).

**Severe anaemia** is defined as haemoglobin concentration both in non-pregnant and pregnant women less than 70 g/L (1).

**Low serum ferritin** (sFt) is defined as sFt <12 µg/L (2).

**High serum transferrin receptor** (sTfR) is defined as sTfR >8.5 mg/L (3).

**Iron deficiency anaemia** is defined in pregnant women by haemoglobin concentration < 110 g/L and presence of low sFt and/or high sTfR.
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Introduction

Anaemia in women

Anaemia is a global public health problem (4). It is defined as a “fall of haemoglobin concentration below a statistically defined threshold laying at two standard deviations below the median of a healthy population of the same age, sex, and stages of pregnancy” (5). Although pregnant women are most frequently affected, it is also prevalent in non-pregnant women and other population groups including children (6). It has been estimated that around 2 billion people in the world are anaemic; most of them are found in low-income countries in Asia and Africa (7).

Anaemia is of great concern particularly during pregnancy because of its reported association with a number of adverse outcomes on health. Broadly speaking, the adverse outcomes of anaemia and haemoglobin concentration have a ‘U-shaped’ association; they occur when maternal haemoglobin values are either at the low or the high end of the range. High haemoglobin concentration in pregnant women is believed to be due to inadequate plasma volume expansion that is associated with a number of pathological conditions including foetal growth retardation (8, 9). At the lower end of the range, haemoglobin concentration has been associated with an increased maternal and perinatal mortality, pre-term delivery and low birth weight (10-16). Infants born to anaemic mothers are also at increased risk of developing anaemia (17, 18). The optimum range of haemoglobin concentrations that has been shown to be associated with a lower risk of intra-uterine growth retardation and pre-term delivery was 96 to 105 g/L (19). In a cohort of Chinese women, the lowest risk of pre-term birth was observed with haemoglobin concentration from 110 to 119 g/L (20). In addition to the adverse pregnancy outcomes, anaemia in women has reportedly been associated with reduced ability to earn income (21), fatigue (22) and impaired immune system (23). Therefore, a reduction of these adverse outcomes is the ultimate goal of strategies aimed at prevention and control of anaemia in women.

Iron deficiency has been claimed to constitute the major part of the anaemia problem. A logical intervention for its prevention and control has therefore been the provision of iron supplementation during pregnancy (1). Although such interventions have been efficacious in controlled settings, they have met with limited success when
implemented as large-scale programmes (24). Many reasons have been identified as to why the existing programmes could not be successful. Some of the major constraints suggested are low accessibility and use of antenatal care, ineffective supply and distribution of supplements, inadequate training and motivation of health care providers, inappropriate and inefficient counselling of mothers, and failure of effective screening and referral procedures (25).

**Conceptual framework**

A conceptual framework of the thesis was developed examining a number of issues that are linked to the aetiology of anaemia and the effect of iron supplementation during pregnancy and puerperium (Figure 1). Firstly, the framework introduces the concepts of iron deficiency and anaemia in pregnant women and relates it to the status before and after pregnancy, hypothesising a circular relationship across the three stages of reproductive cycle, i.e., non-pregnant, pregnant and post partum. Secondly, the model indicates some of the major causes of anaemia. Socio-economic status (SES) has been included as a major associated factor. It is linked to anaemia through three major pathways: diet, parasitic infestations and infections. Lastly, the model assesses the effect of iron supplementation during pregnancy by comparing the two dose frequencies, i.e., daily and weekly, and examines the occurrence of side effects as a potential determinant of compliance. The differential effect of daily and weekly regimens of iron supplementation during pregnancy and puerperium at 6 weeks post partum is also evaluated. The SES and associated cultural factors may modify the perceptions of side effects as well as its influence on compliance.

The model highlights that iron deficiency and anaemia represent two different but interrelated entities and the overlapping area is referred to as iron deficiency anaemia (Figure 1). Depending upon the distribution of different etiologic factors including those other than iron deficiency, the degree of overlap (or the contribution of iron deficiency to anaemia) is expected to differ between geographical areas, socio-economic groups and between different stages of the reproductive cycle within the same population. An increased prevalence of anaemia caused by other factors including infections will, thus, proportionately reduce the anaemia that is caused by iron deficiency.
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Figure 1. Conceptual framework

SES

Associated factors

Diet Parasites Infections

Causal factors

Side

Controlling factors

Iron dose frequency

Compliance

Non-pregnant

Pregnant

Post partum

A

IDA

ID

A

IDA

ID

A

IDA

ID

Early

Late

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Introduction

The model emphasises that SES influences anaemia at all three stages of the reproductive cycle, mainly mediated through diet, parasitic infestations and infections. In this thesis some information on parasitic infestation (hookworm and roundworm) is available, while the occurrence of chronic or acute infections or dietary intake have not been addressed. Therefore, the link between SES and anaemia that has been made is mediated through these three factors. The social and cultural background may influence compliance to iron supplementation and occurrence of side effects. Motivation to adhere to the recommended intake of supplements and also perceptions of side effects may vary with socio-economic status, resulting in differences in compliance.

The effect of iron supplementation is a function of the proportion of anaemia that is attributed to iron deficiency as well as the degree of compliance. Compliance refers to a person’s behaviour in response to a specific medical advice, which may also be termed as adherence (26). Long-term medical regimens including iron supplementation often face problems related to limited compliance. In general, compliance to iron supplementation is affected by a number of factors including side effects, appearance and quality of the supplements, understanding and motivation of health workers and interaction between the supplement providers and users (27). As indicated in the model, compliance is hypothesised to be affected by the dose frequency of iron supplementation and also by the side effects that are experienced due to ingestion of iron compounds. To what extent side effects reduce compliance is still controversial (28, 29). A major part of the controversy is due to unavailability of accurate information on side effects and their impact on compliance in relation to iron supplementation (30). Therefore, a careful assessment of side effects among women in daily and weekly regimens of iron supplementation and its impact on compliance has been studied in this thesis.

A valid measure of compliance is needed to evaluate and compare the efficacy and trial effectiveness of the daily and weekly dose frequencies of iron supplementation. Efficacy is defined as “the extent to which a specific intervention, procedure, regimen, or service produces a beneficial result under an ideal condition” (31). In the present context, it has been interpreted as the extent to which iron tablets ingested either in a weekly or daily frequency produces a differential effect on haemoglobin concentration. The difference in efficacy of the daily and the weekly iron supplementation frequency evaluates the so called “mucosal block” theory (32). The theory advocates that the same dose of iron from
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Tablets administered daily is less efficiently absorbed and incorporated into haemoglobin than iron from tablets taken weekly. In this thesis, however, two types of efficacy analyses have been performed - effect per iron tablet consumed and effect of an entire 12-week regimen of iron supplementation. It is assessed at the end of 12 week iron supplementation during pregnancy and interpreted as the extent to which iron supplements consumed either by daily or weekly frequency produced a differential effect. The aim of the efficacy analyses will, therefore, be to compare biologic differences in the response to iron supplementation between daily and weekly groups.

Effectiveness is a function of efficacy as well as of compliance. It is influenced by both biological and behavioural factors. In the effectiveness analysis compliance is treated as part of the outcome of the intervention. Effectiveness has been defined as “the extent to which a specific intervention, procedure, regimen or service when deployed in the field, does what it is intended to do for a defined population” (31). In the context of this thesis, effectiveness is affected both by efficacy in terms of response to haemoglobin concentration as well as by compliance to daily and weekly regimens of iron supplementation, respectively. Conceptually, programme effectiveness includes, in addition to women’s compliance to iron supplementation, other aspects of the intervention including supplementation policy, coverage, supply of iron tablets and delivery system (27). However, in the present study, all factors except compliance are controlled for and, therefore, what is assessed is the effectiveness of an intervention in a research setting that is referred to as ‘trial effectiveness’. The trial effectiveness was analysed by comparing the response to haemoglobin concentration between women receiving daily and weekly regimens of iron supplementation including the effect of compliance.

Apart from the analysis during pregnancy, the thesis also compares the trial effectiveness at 6 weeks post partum. Since haemodilution or physiological anaemia is pronounced at mid pregnancy (33), it is difficult to interpret the relationship of anaemia between early and late pregnancy and that between late pregnancy and the post partum period. By examining haemoglobin concentration of women receiving daily or weekly dose frequencies of iron supplementation, the thesis is also expected to contribute knowledge in relation to the effectiveness of iron supplementation during pregnancy and puerperium at 6 weeks post partum.
Prevalence of anaemia

Despite that anaemia affects a large number of the world's population, accurate data on prevalence are not available in many low-income countries. In the mid-eighties, data on global prevalence of anaemia were compiled for the first time (34). In low-income countries, 56% of the pregnant and 44% of the non-pregnant women are reportedly anaemic in contrast to 18% and 12%, respectively, in high-income countries (6). Anaemia prevalence is estimated to be even higher among pregnant women in South Asia, being highest in India (87%) (35). The prevalence in pregnancy has been reported to be 59% in Bhutan, 65% in Nepal, and 60% in Sri Lanka (36). In Bangladesh, the estimated prevalence of anaemia is 50% (37). Therefore, it indicates that a large variation in anaemia prevalence exists between countries and possibly within a country.

Although iron deficiency is known to be an important cause of anaemia, its prevalence is not adequately known. Anaemia, as assessed by haemoglobin concentration, has often been used interchangeably to report the number of people with iron deficiency anaemia (7). This has been done partly because the assessment of haemoglobin concentration is relatively cheaper, requires less technical skill and is easier to administer in the community (5). In contrast, although a number of methods are available to assess the level of body iron, most of the methods are costly, require special skills to administer and thus, are not recommended for most of the low-income countries. Therefore, a commonly acceptable definition of iron deficiency is needed that is feasible from both technical and economical points of view.

Since the first publication of DeMaeyer and Adiels-Tegman on the global prevalence of anaemia (34), the same data have been adjusted to the population growth rates for the subsequent projections of anaemic people in the world (1, 38). Further, it has been assumed that for every case of anaemia there would be at least 2.5 cases with iron deficiency. Hence, the 1985 anaemia prevalence estimate was used as the basis to project the total number of people with iron deficiency or iron deficiency anaemia (4, 7, 39-41).

Aetiology of anaemia

The aetiology of anaemia is complex and often a combination of factors. SES is known to be a major associated factor expressing itself
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through diet, parasitic infestations and chronic infections. Deficiencies of one or more nutrients including iron, folic acid, vitamin B12 and vitamin A are proposed as important dietary causes of anaemia (42-47). Among these, iron deficiency is suggested to give the highest attributable proportion (41) and little is known about the relative importance of the other nutrients.

The major causes of iron deficiency include insufficient intake of iron-rich foods and poor bioavailability of consumed iron in relation to the need during pregnancy (39). Poor bioavailability has been reported as the major cause of iron deficiency in a population whose diet is predominantly cereal-based (48). Apart from the form of iron present in the diet (heme and non-heme), factors inhibiting (for example, phytate, calcium) or enhancing (for example, ascorbic acid) its absorption are also important (49). In addition, increased need of iron may also occur through blood loss that may be pathologic, e.g., hookworm infestation (50) or physiologic, e.g., increased need during pregnancy and blood loss during delivery (39).

Other causes of anaemia not linked to dietary deficiency include chronic infections (51), malaria (52-55) and HIV/AIDS (56, 57). Abnormal formation of haemoglobin, e.g., thalassemia and sickle cell disease (39), also cause anaemia in some regions of the world, including Bangladesh (58).

Repeated cycles of reproduction may also increase the risk of anaemia in adult women. Although not clearly shown, closely spaced pregnancies have been suggested to reduce nutritional reserves including iron and, if not properly supplemented, contribute to the subsequent low haemoglobin concentration (59). Haemoglobin concentration in non-pregnant women has been suggested to be one of the predictors of anaemia during pregnancy (60).

Iron deficiency

Iron is used in the body for producing several haeme-compounds including haemoglobin, myoglobin, and cytochromes. Among these, haemoglobin is involved in the delivery of oxygen from the lungs to the tissues and transfer of carbon dioxide from the tissues to the lungs. Oxygen is used by the tissues to produce cellular energy (61). At least two-thirds of the body iron is functional and is found in circulating red blood cells as haemoglobin, in muscle cells as myoglobin and in iron containing enzymes. The rest is stored as ferritin and haemosiderin. The storage iron can be mobilised whenever needed. Women,
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particular during pregnancy, tend to have inadequate storage iron to meet the demands of increased red cell mass and foetal growth. Iron depletion is the decrease of storage iron measured by a reduction in sFt, while an iron deficient erythropoiesis develops in the next stage when the storage iron is depleted and absorbed iron is insufficient to meet the requirements. This stage is characterised by impaired synthesis of and subsequent fall of haemoglobin concentration. Iron deficiency anaemia is characterised by coexistent indications of iron deficiency and anaemia.

Serum ferritin (sFt) has frequently been used to estimate the stored body iron. Its concentration <12 µg/L has been suggested to indicate depleted iron stores (2). However, sFt is an acute phase reactant protein and the concentration is elevated in response to infections. As a result, interpretation of results based on this indicator may be difficult particularly in settings where infections are prevalent (5). In recent years, assessment of soluble serum transferrin receptors (sTfR) has been suggested as a more reliable measure of tissue iron deficiency because it is neither affected by infections nor by the state of pregnancy (62-64). A cut-off of sTfR >8.5 mg/L has been suggested to indicate tissue iron deficiency (3). Apart from the direct measures, an alternative way to assess iron deficiency is to monitor haemoglobin response to iron supplementation (65). A haemoglobin increase of 10 g/L after 1-2 months of the supplementation has been considered as diagnostic of iron deficiency (42). However, while this method is relevant in high-income countries, it may be less useful in low-income countries where several factors of anaemia coexist along with iron deficiency.

Prevention and control of anaemia

Control of anaemia in pregnant women
Distribution of iron supplements has been considered the most appropriate short-term measure to control anaemia. Although folic acid is also provided in combination with iron, little is known about its importance in anaemia prevention and control programmes. The current recommendation of iron supplementation is to provide 60 mg iron and 400 µg folic acid per day to all pregnant women for 6 months in pregnancy and continuing to 3 months post partum when the level of anaemia in a population is more than 40% (66).
Community-based trials both in high- and low-income countries have shown that it is possible to prevent the fall in haemoglobin concentration during pregnancy by providing iron supplements to pregnant women (17, 47, 54, 67-70). However, iron supplementation as a public health measure (e.g., as part of antenatal care programmes) could not be successful in reducing the problem (25). Consequently, the effectiveness of these programmes has been questioned particularly in the context of low-income countries (24). Large-scale iron supplementation programmes that did not demonstrate any significant improvement in haemoglobin concentration include those in Indonesia (71, 72), India (38, 73) and the USA (74). A number of factors have been identified that limit the programme effectiveness. These include lack of an appropriate supplementation policy, inefficient and irregular supply of the supplements, low programme coverage, and low compliance to the recommended supplementation regimen (27, 28, 38, 75). In the present context, the focus was to evaluate the effectiveness of an iron supplementation intervention in a research setting (trial effectiveness) and therefore, operational issues other than compliance were not dealt with.

**Compliance to iron supplementation**

Although compliance has been studied from a wide range of scientific perspectives, until now there is no agreement regarding its common definition (76). The term ‘compliance’ was first introduced in medicine in 1976 (77). Sackett and Haynes later defined compliance as “the extent to which a person’s behaviour (taking medications, following a recommended diet or executing life-style changes) coincides with medical or health advice” (78). ‘Compliance’ or ‘adherence’ may also refer to outcomes of a patient-provider interaction (79). While the term compliance implies the responsiveness by the service recipients, adherence puts more responsibility on the health care providers, which possibly enhances the outcome of a health service (26). In the context of this thesis, the term compliance has been used to refer the extent to which women follow daily and weekly regimens of iron supplementation, respectively. It is calculated as total number of iron tablets taken divided by the number originally recommended during a specified time period multiplied by hundred.

A careful assessment of compliance is necessary to compare the efficacy of two different dose frequencies of the supplementation regimens. Measurement of compliance will also enable an estimation of the total amount of iron that is required to achieve the maximum haematological response. It is important for health personnel to know
the desirable level of compliance and to assess how realistic such a level is to achieve in a community setting.

Poor compliance limits the programme effectiveness (28, 80-82). It has been considered a major problem in iron supplementation trials both in high- (81, 83) and low-income countries (29, 67, 80, 84). In Tanzania, a low level of compliance was observed among pregnant women (29), suggesting that the prescribed dose of iron supplementation often did not correspond to the actual dose taken. Information about compliance is important for the interpretation of the effectiveness of an iron supplementation programme. As mentioned earlier, iron deficiency is not the only cause of anaemia. A programme’s limited effect on anaemia may be due to lack of compliance or alternatively due to a combination of low compliance and a less prominent causal role of iron deficiency. Unless compliance is carefully monitored, it is difficult to separate the two explanations. In recent years, a number of studies have been conducted to compare the effectiveness of iron supplementation programmes with the daily and weekly dose regimens. Unfortunately, the results could not be clearly interpreted due to insufficient information on compliance (85).

Gastrointestinal side effects to iron supplements are often considered to be the main reason for poor compliance (67, 86). However, the relative impact of side effects on compliance has recently been questioned (30). The reason is that a large part of non-compliance is not explained by the occurrence of side effects (29). Most studies evaluating iron supplementation do not report the actual prevalence of side effects and, therefore, their impact on compliance is not possible to assess. Other factors influencing compliance include the appearance and quality of the supplements (66), perceptions and understanding of pregnant women on the rationale of iron supplementation and motivation of the health care providers (87).

In community-based iron supplementation interventions, common methods of assessing compliance include pill count (88), recall of tablet intake (89) and assessment of iron content in stools (81). Pill count and recall of iron tablet intake are quantitative methods providing a rough estimate of the total number of tablets taken. None of these methods is able to assess the day-to-day variation in the tablet intake. However, the methods became popular due to easy applicability and low cost (90). Iron content in stool samples gives a crude judgement of compliance, identifying the proportion of women ingesting iron tablets...
during the past few days. Therefore, it does not quantify the number of tablets taken. Thus, each of these available methods has limitations.

In recent years, a new device has been constructed to assess patient compliance, known as Medication Event Monitoring System, MEMS® (91). It consists of an ordinary pill bottle equipped with a cap, which has an electronic counting device and a small microprocessor embedded. Each time the bottle is opened and closed the time and date are recorded. Use of MEMS® permits collection of continuous information on compliance over an extended period of time.

**Daily and weekly dose frequency**

As stated earlier, the current recommendation of daily iron supplementation has not been as effective as expected to reduce the problem of anaemia during pregnancy. Based on an experiment in rats (32), it was hypothesised that a first dose of iron would load the mucosa and block subsequent doses from absorption. Therefore, by reducing the frequency of supplementation to once per week, matching the mucosal turnover time, iron from each tablet would be better absorbed, the effect per tablet would be higher and consequently a lower amount of iron would be required. This is known as the “mucosal block” theory. According to this theory, it was suggested that absorption of a weekly frequency of iron supplementation would be more efficient as compared to the daily frequency. However, the theory has so far not been supported by human studies. Using radio labelled iron, it was shown that a small reduction in absorption could be achieved due to precedent administration of iron (92). This suggests that the “mucosal block” theory is probably not true in humans and, logically, the daily supplementation frequency should produce a larger haemoglobin response because of the higher amount of iron it provides. Subsequently, a number of community-based trials have been conducted comparing daily and weekly dose frequencies of iron supplementation in pregnant women (80, 92-96). Similar studies have also focused on other population groups, e.g., pre-school children and adolescent girls (97-102). Comparison of results between these studies was difficult since they had variations in the design, answering different research questions. Most of these studies compared final haemoglobin concentration between the supplementation groups without considering the potential differential level of compliance. The studies conducted in Pakistan (93), Malawi (94) and Indonesia (95) did not include any valid measure of compliance, but compared the trial effectiveness between daily and weekly regimens of iron supplementation. This is often referred to as “intent-to-treat” analysis.
Introduction

In contrast, iron tablet intake was supervised in a trial in China (96) and, thus, the efficacy of the intervention was evaluated. Therefore, none of these studies could answer the question if the finding of no difference in trial effectiveness between the supplementation regimens was explained by differential compliance or poor efficacy or a combination of both.

There may be a number of explanations why these community-based trials could not show any difference between the daily and weekly regimens of iron supplementation. Firstly, the population may have responded to a limited extent due to low intake of supplements, lower contribution of iron deficiency than what was expected or presence of causal factors other than iron deficiency such as deficiency of vitamin A (46). Secondly, the two groups of women might have differential – but not measured – levels of compliance. There could be a considerable overlap in the number of tablets taken between the groups, resulting in similar level of intake over the supplementation period. A third explanation may be that the amount of iron provided by the weekly supplementation regimen produced the maximum response and no further increment could be achieved by the additional amount of iron provided by the daily supplementation regimen.
The research project

Objectives

As outlined above, anaemia is common during pregnancy and iron supplementation is globally recommended as a measure for its prevention and control. The objectives of this thesis are to assess the anaemia problem in a rural population of Bangladeshi women, and to compare the impact of a daily and a weekly iron supplementation during pregnancy and puerperium.

The specific objectives are to:

- assess anaemia and iron deficiency in non-pregnant and pregnant women in rural Bangladesh,
- compare the efficacy between daily and weekly frequencies of iron supplementation during pregnancy, and
- compare the trial effectiveness of daily and weekly regimens of iron supplementation during pregnancy and puerperium on haemoglobin in pregnancy and at 6 weeks post partum.
The research setting

*Figure 2. The map of Bangladesh.*

The study was conducted in a rural location of Bangladesh (Figure 2). It is the largest delta in the world formed from the alluvial deposits of three major rivers flowing from north to south. Bangladesh is one of the highest populous countries in the world with an approximate population size of about 127 million and a density of 865 inhabitants per sq. km (103). A major proportion of the population (76%) lives in rural areas (103). The literacy rate is low, 27% for female and 50% for male (103). About half of the total population is poor and a large proportion does not have access to health care (104). One-third of the population cannot meet their daily calorie requirements (105). Around 38% of the women suffer from chronic energy deficiency (BMI <18.5) and weight gain during pregnancy is not more than 5-7 kg (130). Maternal mortality is high, estimated at 350 per 100,000 new born (131). Around 45% of the newborns are of low birth weight (birth weight <2500 g) (106) and the prevalence of stunting and underweight in children under 5 years is 55% and 56%, respectively (107).
Administratively, Bangladesh has 6 divisions, 64 districts and 492 upazilas (sub-district) (103). The upazilas are divided into unions and each union is further divided into villages. Each village has a population of around 1,000. The studies were conducted in rural communities of Mymensingh district, about 110 km north-west of Dhaka city. This area has a high population density; low literacy, high malnutrition and limited access to health services and are, in these regards, comparable to rest of the plains of the country. The diet is dominated by rice, vegetables and some lentils. It is occasionally mixed with pieces of fish and less frequently with meat. Malaria is not endemic and no case of HIV has been reported from the region. It is an area with many BRAC activities and networks, including the antenatal care centres (ANCC).

BRAC, now the largest private development organisation in Bangladesh, was established in 1972 with the main objective of improving socio-economic conditions and health and nutritional status of the rural people. The organisation has, in addition to the rural development programmes and credit schemes, non-formal primary education and health programmes (both preventive and curative) (108). Aside from these programmes, BRAC has a number of support services including monitoring, training and research. The Research and Evaluation Division (RED) is an independent unit of BRAC.

The major activities within BRAC health programmes include early identification and registration of pregnant women, provision of antenatal care, referral of high risk pregnancies and emergency cases, treatment of acute respiratory infections and tuberculosis and provision of centre-based curative medical services. Antenatal care services to rural mothers are provided through the community-based ANCCs. Each ANCC covers around 1,000 people, operated once a month and managed by a BRAC female health staff. She is assisted by a female voluntary health worker who is a permanent resident in the village and popularly known as Shasthyo Shebika (SS). The presence of ANCCs was an important prerequisite in organising the field activities of the iron supplementation trial. Two neighbouring BRAC administrative areas (Dapunia and Samvugonj) in the Sadar upazila of Mymensingh district were selected for the study (Figure 2). There were 54 ANCCs in these two areas.
Design and methods

The thesis is based on results from two studies: (i) a cross-sectional survey of non-pregnant women conducted in February-March 1996, and (ii) a randomised iron supplementation trial in pregnant women conducted from May 1997 to November 1998 (Figure 3).

The cross-sectional survey included a sample of 184 women who were selected through a multistage cluster sampling procedure. The women were aged 15-45 years, married, apparently healthy and reportedly non-pregnant at the time of the survey. Of these, 4 refused finger prick blood sampling and one was later found to be pregnant, leaving 179 women to the final analysis (Paper I).

The iron supplementation trial was conducted on women attending 50 out of a total of 54 ANCCs. The ANCCs were randomly assigned to either daily or weekly doses of iron supplements: 25 to daily and 25 to weekly. The daily supplementation group received one tablet per day and the weekly group two tablets once per week. Each tablet contained 60 mg elemental iron as ferrous sulphate in combination with 250 µg folic acid. The women were recruited based on three inclusion criteria: (i) fundal height from 14 to 22 cm, corresponding to gestational age 18-24 weeks (109), (ii) haemoglobin concentration ≥80 g/L, and (iii) not using any previous iron supplements during the current pregnancy. Baseline information including haemoglobin concentration was available on 214 pregnant women (Paper II), and of these, 209 were included in the iron supplementation trial (daily, n=104; weekly, n=105). Among the women enrolled, 140 (daily n=66, weekly n=74) had complete information on haemoglobin concentration at baseline, at week 4, 8 and 12 and compliance from week 1-11 of the supplementation (Paper III); 172 (daily n=86, weekly n=86) had information on side effects during the first 4 weeks of the supplementation and compliance from week 5-11 (Paper IV); and finally, 146 (daily n=67, weekly n=79) had information on haemoglobin concentration at baseline and at six weeks post partum and compliance from week 1-4 (Paper V) (Figure 3). Major reasons of drop out in papers III-V included delivery before completing the 12-week supplementation regimen, errors in MEMS® resulting in loss of iron tablet intake data, and refusal of blood sampling at 6 weeks post partum.
Figure 3. Study population for the different papers.

**Supplementation trial**
- 611 pregnant women initially registered by household visits for iron
- 214 eligible women gave consent and blood samples taken (Paper II)
- 209 women recruited from 50 ANCCs randomly assigned
  - 3 Hb <80 g/L
  - 2 withdrew consent
- 104 women enrolled in 25 ANCCs assigned to daily regimen
  - 66 women for analysis (Paper III)
    - 23 delivered <12 wk of suppl.
    - 2 withdrawn from participation
    - 2 Hb <75 g/L
    - 11 MEMS failure
- 86 women for analysis (Paper IV)
  - 18 MEMS failure
- 67 women at 6 wk post partum for analysis (Paper V)
  - 21 refused blood sampling at 6 wk postpartum
  - 1 withdrew from trial
  - 15 MEMS failure

**Cross-sectional survey**
- 184 non-pregnant women cross-sectional
- 179 women in paper I
  - 4 refused blood sampling
  - 1 Latter found pregnant
- 105 women enrolled in 25 ANCCs assigned to weekly regimen
  - 74 women for analysis (Paper III)
    - 16 delivered <12 wk of suppl.
    - 1 withdrawn from participation
    - 1 stillbirth
    - 1 lost pill bottle
    - 12 MEMS failure
- 86 women for analysis (Paper IV)
  - 18 MEMS failure
- 79 women at 6 wk post partum for analysis (Paper V)
  - 10 refused blood sampling at 6 weeks postpartum
  - 2 withdrew from trial
  - 14 MEMS failure
Below a brief methodological overview is provided of papers included in the thesis.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Subjects</th>
<th>n</th>
<th>Time period</th>
<th>Data source</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional</td>
<td>Non pregnant women 15-45 years, apparently healthy</td>
<td>179</td>
<td>February to March 1996</td>
<td>Questionnaire Haemoglobin Parasites in stools</td>
<td>I</td>
</tr>
<tr>
<td>baseline in community-based trial</td>
<td>Pregnant women, 15-45 years, 2nd trimester</td>
<td>214</td>
<td>May and November, 1997</td>
<td>Questionnaire Haemoglobin Iron status Parasites in stools</td>
<td>II</td>
</tr>
<tr>
<td>Randomised community-based trial</td>
<td>Pregnant women, 15-45 years</td>
<td>140</td>
<td>May 1997 to January 1998</td>
<td>Questionnaire Haemoglobin Iron tablet intake</td>
<td>III</td>
</tr>
<tr>
<td>Randomised community-based trial</td>
<td>Pregnant women, 15-45 years</td>
<td>172</td>
<td>May 1997 to January 1998</td>
<td>Questionnaire Side effects Iron tablet intake</td>
<td>IV</td>
</tr>
<tr>
<td>Randomised community-based trial</td>
<td>Pregnant women, 15-45 years, followed to 6 weeks post partum</td>
<td>146</td>
<td>May 1997 to November 1998</td>
<td>Questionnaire Haemoglobin Iron status Iron tablet intake</td>
<td>V</td>
</tr>
</tbody>
</table>

$sFt = Serum ferritin$, $sTfR = Serum transferrin receptors$

**Design**

The cross-sectional survey: The survey was conducted in 12 villages in Fulbaria upazila of Mymensingh district. The selection of the upazila was done to represent an area covered by the governmental reproductive health services. With respect to health, nutritional, demographic and socio-economic indicators, the area is comparable to the rest of the rural plains of Bangladesh. Twelve villages were selected randomly from a complete list of all villages in the upazila. The interviewers performed house-to-house visits from the centre of the village and then moved in one direction until the recruitment of 15
women per village was completed. This sampling method has been recommended in health studies in low-income countries (110).

**The iron supplementation trial:** A total of 611 pregnant women in the area covered by the 50 ANCCs were initially registered through house-to-house visits (Figure 3). During the registration, information on age, parity, gestational age and SES was collected from all identified pregnant women. They were encouraged to receive the antenatal care service from the respective centres on a fixed date. On the day of the scheduled antenatal service, the first 4 women who arrived at the centre and fulfilled the inclusion criteria were included in the study. Blood samples were taken from women who had a fundal height between 14-22 cm, not receiving iron supplements during the pregnancy prior to the trial and gave consent to participate. The venous blood sample was collected in an untreated evacuated tube. Thus, the baseline blood samples were taken from 214 women and their haemoglobin concentration was assessed. Of these, 3 women had haemoglobin concentration <80 g/L and 2 withdrew their consent right after the first blood sampling, leaving 209 pregnant women for the final enrolment in the trial. During the course of the trial, women’s haemoglobin values were assessed monthly on a drop of capillary blood. If a single measurement fell below 75 g/L or the level of two repeated monthly examinations fell between 75 and 79 g/L, women were excluded from the study, transferred to daily supplementation and referred to a BRAC health centre (3 women were excluded due to fall in haemoglobin concentration). Women assigned to the daily regimen received a MEMS® pill bottle with 100 tablets and were advised to take one tablet every day of the week. The weekly group received a MEMS® pill bottle with 30 tablets, and they were advised to take 2 tablets, one in the morning and one in the evening, every Friday. The women returned the MEMS® pill bottles after 12 weeks of supplementation, including any remaining tablets. For the rest of the study duration (until 6 weeks post partum), iron supplements sufficient for the daily and weekly groups, respectively, were provided in the same bottles replacing the MEMS® with ordinary caps.

**Methods**

**Haemoglobin concentration:** Finger prick blood samples were used to assess haemoglobin concentration in non-pregnant women. From each woman, 20 µl of capillary blood samples were collected and preserved in a vial containing 5 ml of Drabkin’s solution. The samples were kept in a cold flask and then transported to Mymensingh Medical College
Hospital laboratory for analysis. Haemoglobin concentration was assessed by the cyanmethaemoglobin method following a standard laboratory technique (111).

Venous blood samples were used to assess haemoglobin concentration at baseline and at 12 weeks of iron supplementation during pregnancy and also at 6 weeks post partum. Capillary blood samples were, however, used to assess haemoglobin concentration at 4 and 8 weeks of the supplementation. At all these occasions, the assessment was done in the community using HemoCue® portable system. The system has been shown to have an accuracy and precision similar to the standard cyanmethylaemoglobin method (112). The accuracy of HemoCue® was checked daily by the use of control cuvettes provided with the machines.

A skilled laboratory technician collects veni-puncture venous blood samples with assistance from an interviewer

Iron status: Iron status was assessed in pregnant women at baseline, 12 weeks of supplementation and at 6 weeks post partum. However, in the thesis, data on iron status at baseline during pregnancy and at 6 weeks post partum are presented. Within 4 hours of collection, the blood samples were transported on ice to a field laboratory. After centrifugation, serum was taken off and frozen temporarily at -20°C for later storage in Dhaka at -70°C. Analyses of the serum samples were performed at the end of the study at Department of Nutrition,
University of California Davis, USA. sFt was assessed using radio immunoassay (Diagnostic Products, San Diego, CA). Soluble sTfR was assessed by an enzyme-linked immunosorbent assay (Ramco Laboratories, Houston, TX).

Iron tablet intake assessment: Compliance to the prescribed dose of iron supplementation was assessed by use of the Medication Event Monitoring System, MEMS® (Apex Corporation, Fremont, California). The intake of iron tablets provided from week 1-12 during pregnancy was monitored. The MEMS® pill bottle is an ordinary bottle fitted with a cap that has a microprocessor embedded. Each event of bottle opening and closing is recorded as a presumptive dose. On the day of iron tablet distribution, all women were instructed how to open and close the bottles. The women were not informed about the real use of MEMS® to minimise its effect per se on their usual tablet taking behaviour.

A special reading device connected to a personal computer retrieved data from MEMS®. Information was provided as listings of date and time of individual bottle opening and closing events and the time in hours since the last dose of an iron tablet taken in calendar plots. Every calendar plot represented 24 hours and numerically showed the number of doses taken during the whole supplementation period. Each
event was considered as a proxy of one tablet taken. The bottle opening and closing events that had occurred the first and the last days were not used in the analysis because they did not provide information on a full day and also some additional events were recorded on the first centre date during demonstration to each woman.

**Fundal height:** Gestational age was determined by measuring fundal height, which was done during the first household visit and was confirmed later on the day of recruitment by specially trained interviewers. Women were asked to empty the bladder and lie in the supine position with legs extended. The measurements were taken in cm along the longitudinal uterine axis using a standard plastic tape. Women with fundal height 14-22 cm, reportedly corresponding to gestational age 18-24 week (109), were included in the study.

![A trained interviewer is measuring fundal height](image)

**Socio-economic status (SES):** House-to-house interviews were held to collect information on demographic, reproductive and SES in both non-pregnant and pregnant women using a structured questionnaire. The women recalled their age and parity. The SES information included attendance at a formal school, perceived household economic status, and household landholding. These indicators have been previously tested and found valid in the rural context of Bangladesh (113). Women with at least one year of completed formal schooling were categorised as having attended school. Women who reported to be economically surplus or balance as opposed to economically deficit in certain occasions or always were categorised as not deficit. The third
indicator of SES included household landholding. Women with households having 0.5 acre or more land were categorised as functionally not landless. The poverty alleviation programmes in Bangladesh commonly use this definition of landlessness to target the poor households (114). A SES score was constructed adding the 3 positive attributes, which ranged from 3 (highest) to 0 (lowest). For example, a woman who reported that she had formal schooling, was economically surplus, and had land, was given a SES score of 3.

**Stool examination:** A plastic container to collect a sample of faeces was given to each woman. The containers with stool samples were picked up through house-to-house visits on the following day. The stool samples were diluted with sodium chloride solution and examined for presence of ova of hookworm and roundworm by a trained laboratory technician. The microscopic examination was performed using a semi-quantitative technique (115). Stool samples were examined to assess the presence of roundworm and hookworm infestations.

**Quality control**
The author was full time responsible for co-ordination of the fieldwork. A full-time field supervisor, who had several years of experience in organising surveys on health and nutrition, directly supervised the daily activities. Six female interviewers, each responsible for 4-5 ANCCs, conducted the field activities. All of them had a Bachelors degree in social science and had at least 2 years of field experience in health and nutrition related data collection. The field workers received three-week training on interview techniques, finger-prick blood sample collection, maintenance of the HemoCue® system and MEMS®, assessment of haemoglobin concentration on capillary and venous blood, safe transport of blood samples from field to the local laboratory, centrifuge of blood samples, separation and storage of serum and assessment of fundal height and anthropometric measurements. The training included both classroom exercises and field practices. Two lecturers of the Haematology Department in Mymensingh Medical College and Hospital provided training on finger prick including assessment of haemoglobin concentration using the HemoCue® system. The training on measurement of fundal height was provided at the Obstetrics unit of the hospital by an expert obstetrician followed by rigorous field practices. The measurements including height, weight, fundal height and haemoglobin concentration were standardised and both inter- and intra-interviewer variations were assessed during the training. Weekly staff meetings were held with all members including the field co-ordinator to review the study progress and to discuss activities.
performed in the previous week. Re-training on all the measurements was conducted at the middle of the study. The field supervisor travelled across the field area every day to monitor the study related activities.

All questionnaires were checked for correctness at two stages. The field supervisor checked and signed all finalised and error-free questionnaires. Any questionnaire with a suspected error was sent back to the respective interviewer for corrections. The field co-ordinator reviewed all the questionnaires that were checked and signed by the supervisor. Any inconsistency was discussed during the weekly meetings for clarifications and, if needed, the questionnaires were sent back to the field for additional corrections. All completed questionnaires were sent to BRAC head office in Dhaka and a trained computer operator entered data into a SPSSWIN database (version 7.5.1).

Statistical analysis

The sample size calculations of the iron supplementation trial were based on haemoglobin estimations in non-pregnant women obtained through a pilot study in the same area. Among women with a haemoglobin concentration ≥80 g/L, the mean concentration was 112 g/L and the variance was 225. A difference between two intervention groups of 5 g/L in haemoglobin concentration was considered of biological significance. Given these prerequisites a sample size of 70 women in each group would suffice for demonstrating a significant (p=0.05) difference. Fifty clinics were randomly assigned to prescribe either daily or weekly frequency of iron supplementation. According to the pilot study, the distribution of haemoglobin concentration did not differ much between the villages (and thus between clinics) in the area. The design effect was thus considered to be limited. However, a 20% increase was considered to be needed for the multivariate character of the final analysis and another 20% increase of the sample size to allow dropouts during the whole course of the trial. Thus, 100 women were required in each of the two dose frequencies leading to a sample of 200 women, distributed over 50 clinics.
Below a summary is provided of research questions and statistical methods that are used in the thesis.

<table>
<thead>
<tr>
<th>Research questions</th>
<th>Analysis/statistical tests</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the prevalence and severity of anaemia in non-pregnant women?</td>
<td>Distribution of Hb.</td>
<td>I</td>
</tr>
<tr>
<td>Is there any socio-economic difference in the prevalence?</td>
<td>Hb distribution by SES/Chi-square test for trend.</td>
<td></td>
</tr>
<tr>
<td>What is the prevalence of iron deficiency and anaemia in pregnant women?</td>
<td>Distribution of sFt, sTfR and Hb.</td>
<td>II</td>
</tr>
<tr>
<td>What proportion of anaemia is due to iron deficiency?</td>
<td>Distribution of sFt and sTfR by Hb/Chi-square test for trend.</td>
<td></td>
</tr>
<tr>
<td>Is there difference in effect per iron tablet taken between daily and weekly frequency?</td>
<td>Predicted Hb response per tablet given daily or weekly linear regression analysis.</td>
<td>III</td>
</tr>
<tr>
<td>Is there difference in effect between a 12-week daily and weekly regimens of iron supplementation?</td>
<td>Predicted efficacy of a 12-week daily and weekly regimens from scatter plot fitting of Lowess curve.</td>
<td></td>
</tr>
<tr>
<td>Is a weekly regimen of iron supplementation more effective than daily?</td>
<td>Hb distribution before and after iron supplementation by daily and weekly/repeated measures and hierarchal ANOVA.</td>
<td></td>
</tr>
<tr>
<td>Is there any difference in compliance between daily and weekly groups?</td>
<td>Cumulative distribution of mean compliance by daily and weekly groups.</td>
<td>IV</td>
</tr>
<tr>
<td>Do side effects differ between daily and weekly groups?</td>
<td>Occurrence of side effects by groups/Chi-square tests.</td>
<td></td>
</tr>
<tr>
<td>Do side effects have an impact on compliance to iron supplementation?</td>
<td>Mean compliance by side effects stratified by daily and weekly groups/Chi-square tests, ANOVA.</td>
<td></td>
</tr>
<tr>
<td>Is there any difference in trial effectiveness between daily and weekly at 6 weeks postpartum?</td>
<td>Hb distribution at baseline and at 6 weeks postpartum/ independent t-tests.</td>
<td>V</td>
</tr>
<tr>
<td>Is there any response to iron supplementation at 6 weeks postpartum?</td>
<td>Distribution of Hb at 6 weeks postpartum by tablet intake categories/ANOVA.</td>
<td></td>
</tr>
</tbody>
</table>

H=Haemoglobin, sFt=Serum ferritin, sTfR=Serum transferrin receptors.
Means and standard deviations were used as a measure of central tendency in the case of normally distributed values including haemoglobin concentration. Lowess (116) smoothed plots of haemoglobin concentration at weeks 4, 8 and 12 of the supplementation were used to visualise dose response relations. Original values of haemoglobin concentration were used to perform all statistical tests. sFt and sTfR were not normally distributed, thus, logarithmic transformation was performed. Medians were used as a measure of their central tendency and transformed values were used in performing the statistical tests. Statistical significance of associations between anaemia prevalence and an individual indicator of SES was analysed by chi-square test. Trend and departure from linearity were also tested by chi-square. To detect difference between two independent groups, student’s t-test was used. Paired t-test was used to detect changes from baseline to 6 weeks postpartum. Relationship between haemoglobin concentration and categorical variables was as well as compliance and side effects was tested by analysis of variance (ANOVA). Multiple linear regression analysis was used to study the difference in efficacy between the daily and weekly dose frequencies of iron supplementation. Stratified analysis was performed to study the impact of side effects on compliance to iron supplementation between different SES groups. Statistical significance was set at p <0.05. For data analysis, SPSSWIN 9.0 statistical package was used.

Potential confounders were identified as those variables with a p <0.20 for any linear or non-linear association with haemoglobin outcomes and either tablet ingestion (for efficacy analysis) or across the daily-weekly regimens (for effectiveness analysis). Confounding by the measured variables was discarded as an important contribution to the main effects if the interactions of their linear or non-linear introductions did not change by more than 10% the parameters describing the dose response or describing the daily-weekly interactions with the regimen.

**Ethical considerations**

The protocols were discussed with the field level BRAC health workers, village health workers and local village representatives. Informed consent was obtained verbally from participating women due to high rate of illiteracy. All participating women were informed about the objectives of the study and how it was going to be conducted. It was emphasised that participation was voluntary and that women were free to leave the study at any time without any further consequences for the
provision of existing health services. The actual function of MEMS® was not disclosed because it had important implication on women’s usual behaviour related to the intake of iron tablets.

The intervention consisted of provision of iron supplements to pregnant women. The supplement had ferrous sulphate as the active compound, which is a standard medication in iron supplementation programmes. Two different dose frequencies of iron supplementation were applied, the daily and the weekly. The supplementation frequencies used the doses of iron that were similar to the doses applied in previous studies co-ordinated by WHO. A specific cut-off level of haemoglobin concentration for exclusion was applied. In the studies conducted elsewhere by WHO, a cut-off of 70 g/L was used. In this study, however, a higher cut-off level (80 g/L) was used. Among the participating pregnant women, haemoglobin concentration was monitored throughout the trial period. Women with low haemoglobin were referred to nearest BRAC health centre for proper diagnosis and treatment. Stool examination was performed at baseline to assess the presence of hookworm, roundworm and whipworm. Although the plan was to treat them according to the WHO recommendation during pregnancy, the drug was provided after childbirth as per the suggestion of the local medical authorities.

The studies included collection of blood samples. Sampling of blood was done both by finger prick and veni-puncture. Trained laboratory technicians performed the veni-puncture, while the interviewers did the finger prick after receiving a standardised training. It was done under hygienic conditions using disposable equipment. The sampling and handling of blood were routinely checked for safety. The results of haemoglobin concentration and stool examinations were reported back to the women. Data were kept confidential and used only for research purpose. The privacy of the participating women was secured by interviewing them in seclusion.

The study protocols were reviewed and approved by the Research Ethics Committee of the Medical Faculty at Umeå University, Sweden and the Ethical Board at Bangladesh Medical Research Council (BMRC), Dhaka, Bangladesh.
Results

Prevalence of anaemia

*Haemoglobin concentration (Anaemia) (Paper I and II)*

Haemoglobin distribution was similar in the non-pregnant and pregnant samples of women (Table 1). However, when applying the recommended cut-offs for anaemia in non-pregnant (<120 g/L) and pregnant women (<110 g/L) (1), the proportion of women classified as anaemic was significantly higher in non-pregnant (73%) compared to pregnant women (50%, \( p<0.001 \)). Very few women were found with severe anaemia.

<table>
<thead>
<tr>
<th>Haemoglobin (g/L)</th>
<th>Non-pregnant n=179</th>
<th>Pregnant n=214</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;70</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>70-99</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>100-109</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>110-119</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>120+</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>Mean</td>
<td>112</td>
<td>110</td>
</tr>
<tr>
<td>SD</td>
<td>18</td>
<td>14</td>
</tr>
</tbody>
</table>

*Iron status (Paper II)*

In pregnant women, medians (25\(^{th}\), 75\(^{th}\) percentiles) of sFt and sTfR were 13.9 µg/L (5.7, 29.5) and 6.2 mg/L (4.4, 8.5), respectively. Prevalence of iron deficiency showed a large variation depending upon the indicator used. Low sFt was found in 42% and high sTfR in 25% of these pregnant women. When iron deficiency was defined as either low sFt and/or high sTfR, the prevalence was 54% and the corresponding prevalence of iron deficiency anaemia was 33%. Using the most conservative indicator of iron deficiency, i.e., coexistence of low sFt and high sTfR, the prevalence of iron deficiency was 13% and iron deficiency anaemia was 10%. The prevalence of iron deficiency based on different definitions revealed that low sFt, high sTfR or both were present in a large proportion of anaemic women, and though less frequently, also in non-anaemic women (Figure 4).
**Results**

**Figure 4. Prevalence of iron deficiency using different definitions in anaemic and non-anaemic women (n = 214).**

**Haemoglobin and iron status (Paper II)**

The proportion of pregnant women with a combination of both low sFt and high sTfR decreased with increasing haemoglobin concentration, and was only found in those with haemoglobin concentration <119 g/L. The proportion of low sFt showed a similar trend and decreased with increasing haemoglobin concentration. However, the proportion of pregnant women with low sFt increased significantly ($p<0.05$) in the highest range of haemoglobin (Figure 5).

**Figure 5. Percentage of women with low sFt (<12 µg/L) at different haemoglobin concentrations levels (n = 214).**

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-29-
Results

Anaemia and socio-economic status (Paper I and II)

In non-pregnant women, anaemia prevalence decreased with increasing SES score. The highest prevalence was observed in women with the lowest SES score (88%), being significantly higher than any of the other socio-economic groups \( (p<0.01) \). Mild anaemia showed a decreasing trend with increasing SES, but was common even in women with the highest SES score (37%). The prevalence of moderate anaemia did not significantly differ between SES groups. There were only two women with severe anaemia - both were found in the lowest SES group (Table 2).

In contrast to non-pregnant women, anaemia prevalence in pregnant women had no association with SES score after controlling for potential confounding factors including age, parity and fundal height.

Table 2. Distribution of haemoglobin concentration, n (%), among non-pregnant women in a rural area of Bangladesh by SES score (n=179).

<table>
<thead>
<tr>
<th>SES Score</th>
<th>0 (lowest)</th>
<th>1</th>
<th>2</th>
<th>3 (highest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb concentration (g/L)</td>
<td>n=58</td>
<td>n=53</td>
<td>n=36</td>
<td>n=32</td>
</tr>
<tr>
<td>120+ (Normal)</td>
<td>7 (12)</td>
<td>15 (28)</td>
<td>14 (39)</td>
<td>13 (41)</td>
</tr>
<tr>
<td>100-119 (Mild anaemia)</td>
<td>34 (59)</td>
<td>30 (57)</td>
<td>16 (44)</td>
<td>12 (37)</td>
</tr>
<tr>
<td>70-99 (Moderate anaemia)</td>
<td>15 (26)</td>
<td>8 (15)</td>
<td>6 (17)</td>
<td>7 (22)</td>
</tr>
<tr>
<td>&lt;70 (Severe anaemia)</td>
<td>2 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Hb = Haemoglobin concentration, SES = Socio-economic status

Effect of iron supplementation

The efficacy of daily and weekly dose frequencies (Paper III)

The mucosal block theory was tested by comparison of response per tablet taken in a weekly and daily dose frequency, respectively. To avoid a potential negative finding due to limited response to iron supplementation, women with a haemoglobin concentration <115 g/L were selected for the analysis. Haemoglobin concentration at 4 weeks...
was plotted against number of iron tablets taken during initial 3 weeks and a Lowess moving average curve was fitted. The curve showed a sharp dose-response relationship up to 15 tablets taken in both daily and weekly frequencies and final analysis was further restricted to those who took 15 or fewer tablets during the first 3 weeks. In a least square regression analysis, the differential effect per tablet was tested by modelling haemoglobin concentration at 4 weeks as a function of initial haemoglobin concentration, number of tablets taken, supplementation group and interaction between groups and number of tablets. The regression model indicated that for each of the first 15 tablets taken, haemoglobin concentration was increased by 1.2 g/L ($p<0.05$) and there was no difference in response per tablet between the daily and weekly dose frequencies ($p=0.54$). Therefore, there was no evidence for the “mucosal block” theory.

**Iron tablets required to achieve maximum effect (Paper III)**

This analysis estimated the total number of iron tablets required to achieve the maximum haemoglobin response during pregnancy. The same sub-set of women with low haemoglobin concentration (<115 g/L) at baseline was included in the analysis. A dose response relationship was observed between haemoglobin concentrations at weeks 4, 8 and 12 and number of iron tablets taken during week 1-3, 1-7 and 1-11, respectively. All these curves were superimposed (Figure 6), indicating that the main determinant of the effect was the number of tablets taken, not the duration of the supplementation. The figure suggested that a sharp haemoglobin response was achieved up to 20 tablets taken. The haemoglobin concentration continued to rise and reached a plateau at around 40 tablets (Figure 6). Dose-effect was compared between women who took less than 40 tablets and 40 or higher number of tablets. A categorical variable was constructed dividing the women in two groups: 39 or less tablets taken (n=56) and 40 or more tablets taken (n=34). Haemoglobin concentration at 12 weeks was modelled as a function of initial haemoglobin, total number of tablets taken during 1-11 weeks, the 40-tablet categorical variable and the interaction between number of tablets taken and the categorical variable. An interaction was found, demonstrating a differential response between the first 39 and higher tablets taken ($p<0.05$); an initial dose-effect followed by a plateau. The analysis showed that 40 iron tablets were required to produce the maximum response in haemoglobin concentration and no additional response was attained after ingestion of any additional tablets.
Results

Figure 6. Haemoglobin concentration (Hb) at week 4, 8 and 12 as a function of tablet intake at week 3, 7 and 11. Lowess moving average fitted line. Subset with initial haemoglobin $<115$ g/L.

Predicted efficacy of daily and weekly dose regimens (Paper III)
The comparison of efficacy of dose regimen refers to the attained haemoglobin concentration that can be predicted over the whole duration of the supplementation period if the compliance had been 100%. The response was predicted from the dose effect curve (Figure 6). If compliance had been 100%, women in the daily and weekly groups would have taken 77 and 22 tablets in 11 weeks, respectively, corresponding to attained haemoglobin concentration of 121 g/L and 115 g/L. Compared to the baseline, the predicted increment in haemoglobin concentration would be 17.1 g/L in the daily group and 11.7 g/L in the weekly group. The predicted increment in the weekly group would then be 68% of that of the daily, suggesting that a 12-week daily iron supplementation regimen would be more efficacious.

Side effects and compliance (Paper IV)
Gastro-intestinal side effects including heartburn, nausea, vomiting, diarrhoea, or constipation were recalled by the pregnant women after the first month of supplementation. Vomiting was reported less
Results

frequently in the daily (10%) than weekly group (21%, \(p=0.02\)), while recall of other side effects did not differ between the groups. Regardless of side effects, compliance was better in the weekly group (104%) compared to that of daily group (69%, \(p<0.001\)). Of the different side effects, vomiting and nausea predicted a subsequent lower compliance but only among women with lower SES.

**Trial effectiveness of daily and weekly dose regimens**

*(Paper III and IV)*

The trial effectiveness analysis makes a comparison between the daily and weekly regimens of iron supplementation disregarding level of compliance. This type of analysis is often referred to as ‘intent-to-treat’ analysis. At baseline, there was no significant difference in haemoglobin concentration between daily and weekly regimens (Table 4). In the full sample of women, a small but significant \((p<0.05)\) difference was found in attained haemoglobin after 12 weeks of supplementation between daily (120 g/L) and weekly (117 g/L) regimens using GLM repeated measures analysis. The difference was more pronounced in a sub-set of women with initial haemoglobin <115 g/L (Table 4). The large trial effectiveness of the daily regimen was confirmed in hierarchical ANOVA taking the cluster randomisation into account.

At 6 weeks post partum haemoglobin concentration had significantly increased compared to baseline in both the daily and weekly iron regimens. However, neither the increment nor the attained level of haemoglobin concentration at 6 weeks post partum differed between the supplementation groups (Table 4). To ascertain if the supplementation during pregnancy and puerperium could produce any effect after childbirth, haemoglobin concentration at 6 weeks post partum was compared by iron tablet intake during pregnancy. Total number of iron tablets taken during the first 4 weeks of the supplementation was divided into three equally sized groups: low (up to the 33rd percentile), medium (33rd to 66th percentile), and high. After adjusting for confounding factors the haemoglobin concentration showed a step-wise increment from low to high tablet intake groups \((p=0.06)\), suggesting a dose dependent relationship at 6 weeks post partum.
Table 3. Haemoglobin concentration at different stages of pregnancy and at 6 weeks post-partum. A sub-set of women with baseline haemoglobin concentration <115 g/L.

<table>
<thead>
<tr>
<th></th>
<th>Daily</th>
<th>Weekly</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>During pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin at baseline</td>
<td>104+8</td>
<td>103+8</td>
<td>0.73</td>
</tr>
<tr>
<td>Increment 4 week</td>
<td>8+10</td>
<td>5+9</td>
<td>0.16</td>
</tr>
<tr>
<td>Increment 8 week</td>
<td>13+12</td>
<td>9+9</td>
<td>0.05</td>
</tr>
<tr>
<td>Increment 12 week</td>
<td>17+13</td>
<td>12+11</td>
<td>0.04</td>
</tr>
<tr>
<td>Attained 12 week</td>
<td>120+15</td>
<td>115+12</td>
<td>0.05</td>
</tr>
<tr>
<td>At 6 weeks post-partum</td>
<td>n=46</td>
<td>n=44</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin at baseline</td>
<td>103+9</td>
<td>104+8</td>
<td>0.39</td>
</tr>
<tr>
<td>Increment at 6 week postpartum</td>
<td>26+17</td>
<td>22+17</td>
<td>0.32</td>
</tr>
<tr>
<td>Attained at 6 week postpartum</td>
<td>127+17</td>
<td>126+16</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**Trial effectiveness in controlling anaemia**

Trial effectiveness of iron supplementation during pregnancy and puerperium in controlling anaemia was compared between daily and weekly regimens (Table 5). All women who had information on haemoglobin concentration at baseline, weeks 4 and 12 and at 6 weeks post partum were included in this analysis (n=135). There was no difference in anaemia prevalence in daily and weekly regimens neither after 12 weeks supplementation nor at 6 weeks post partum (Table 5). Nevertheless, after first 4 weeks of iron supplementation, there was a 20% reduction of anaemia in women assigned to daily regimen compared to only 8% in weekly regimen suggesting that daily iron supplementation regimen treats anaemia faster.

Table 4. Impact of iron supplementation during pregnancy and at 6 weeks post partum in relation to iron supplementation regimen (daily, weekly).

<table>
<thead>
<tr>
<th>Supplementation regimen</th>
<th>n</th>
<th>Anaemia prevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Daily</td>
<td>59</td>
<td>51</td>
</tr>
<tr>
<td>Weekly</td>
<td>76</td>
<td>46</td>
</tr>
</tbody>
</table>

Wk=week, PP=Post partum
Discussion

Main findings

Anaemia was prevalent in both pregnant and non-pregnant women but not of the severe type. The prevalence of iron deficiency anaemia during pregnancy ranged from 10% to 33% depending on the indicator used to define iron deficiency. There was no increased haemoglobin response of iron supplements taken in a weekly dose frequency compared to daily during pregnancy. The daily regimen produced a larger response after 12 weeks of the supplementation due to the larger number of iron tablets it provided. Over the 12-week period during pregnancy, 40 tablets were sufficient to produce the maximum haemoglobin response. Side effects occurred to a similar extent in both the daily and weekly regimens, except for vomiting that had a larger prevalence in weekly group. Compliance was reduced in women experiencing vomiting, but the overall effect of side effects was limited. At 6 weeks post partum, there was no difference in haemoglobin concentration between the daily and weekly regimens.

Validity

The prevalence estimates

Non-pregnant: The selection of the non-pregnant women was done through a cross-sectional survey based on a cluster sampling procedure (110). The villages were randomly selected and the selection of women from each village was done systematically. The design provided an equal opportunity to all the eligible women to be included in the sample. Two factors might have limited women’s enrolment: availability at home and consent to participate. The cultural and livelihood practices in rural Bangladesh are such that most of the activities that the women are responsible for take place near their houses. Thus, it is not likely that women were unavailable to an extent that could have biased the sample. Another potential source of omission was refusal of the women to participate. However, the experience from the field and reports from interviewers suggested a low rate of such refusals.

Pregnant: The community-based health workers of BRAC initially identified the pregnant women as part of their routine programme
activity. The experience of the programme is that early pregnancies are often missed and most pregnant women are identified late in their first, or early in their second trimester. After a pregnant woman was identified, she was visited at her house by one of the interviewers to confirm the pregnancy and to control the inclusion criteria of fundal height < 22 cm. For women fulfilling the inclusion criteria, baseline characteristics including demographic, socio-economic and reproductive history were collected. Thus, the procedure to identify a pregnant woman and the further selection criteria made the selected sample to mainly consist of women in their second trimester. Thus, data on haemoglobin concentration and iron status are not reflecting all stages of pregnancy, but focus on the second trimester. The effect of haemodilution is most pronounced in the second trimester (117). As a result, it may be that this sample of pregnant women overestimates the problem of anaemia during pregnancy. It may, however, provide a better description of the period in pregnancy where the most severe anaemia can be expected.

Baseline information was collected on all identified pregnant women (n=611) in the catchment area of the 50 ANCCs. This enabled a comparison between women later enrolling in the study (n=214) and those who did not (n=397). Parity was higher among enrolled women (p<0.05), but no other baseline characteristics differed between the groups (Paper II). Parity had a negative association with initial sFt (p=0.03), but not with haemoglobin concentration or sTfR. Each additional pregnancy was associated with a mean of 4 µg/L lower sFt concentration. Accounting for difference in parity between women enrolled and not enrolled, it can, thus, be expected that the women who were enrolled had on average 1.6 µg/L lower sFt concentration, which would not bias the estimate to a significant extent.

Lost to follow up in the trial
Pregnant women with incomplete information had a higher fundal height at baseline, indicating a more advanced gestational age at the beginning of the supplementation than those with complete information (Paper III). This occurred to a similar extent in both the daily and weekly regimens. As there was no difference in fundal height between the daily and weekly groups among women with complete information, it is unlikely that fundal height confounded the analysis. Significantly more women in the weekly group with low haemoglobin had incomplete information. Exclusion of these women might have resulted in both an over- and underestimation of the response in the weekly group. The increment in haemoglobin response could be
underestimated as these women were expected to have a larger response, while an overestimation in the attained haemoglobin could occur as the women were expected to have had lower haemoglobin at week 12. However, as the initial haemoglobin concentration in the rest of the women who were included in the analysis did not differ between the supplementation groups, it is unlikely that the results were biased to any significant extent.

A comparison between women enrolled and lost to follow up at 6 weeks post partum assessment did not show the same pattern regarding fundal height (Paper V). In contrast, a greater loss of women with lower fundal height was observed in the daily regimen \( (p=0.01) \) as compared to the weekly. However, fundal height did not differ between the groups and, therefore, could not bias the comparison at 6 weeks post partum to any significant extent.

**Confounding factors**

The main outcome variable in most of the analyses presented in the thesis is haemoglobin concentration. It is known that haemoglobin concentration can be associated with age, parity, gestational age, SES and parasitic infestations. These factors, therefore, could potentially confound the results. The risk of confounding was fundamentally different in the efficacy and the trial effectiveness analyses. In the latter analysis, the intervention (daily vs. weekly regimen) was randomised and the potential confounding factors should, thus, be equally distributed in both the groups. Still, all analyses were tested for the confounding effect, but there was no evidence that the results were confounded.

In the comparison of efficacy between the daily and weekly regimens, the main determinant tested was the amount of iron supplements consumed (Paper III). Number of iron tablets actually consumed was, thus, not randomised and there was a potential risk for confounding. In all the efficacy analyses, the potential confounding factors were tested for the association to tablet intake as well as haemoglobin outcome. In analyses where there was a risk for confounding, it was tested whether it had an impact on the estimates. However, there was no evidence of confounding in the efficacy analyses.

**Assessment of tablet intake and side effects**

Information on number of pill bottle opening events by MEMS® was used as an indicator of tablet intake. Events recorded on the first and last days were excluded since a full day was not monitored and because
extra openings might have occurred when women returned home from the ANCC and wanted to show the content of the bottle. While it is possible that the women might have opened the pill bottle and not consumed a tablet, it is not likely that it happened to a large extent. The women were not informed about the monitoring function of MEMS® and, thus, there should be no reasons for them to open the bottles in a systematic way to pretend that they had taken the tablets.

The calendar plots that were generated after retrieving data from MEMS® did not only provide the number of openings, but also the pattern of openings over weeks. These patterns clearly indicated that women took the tablets routinely, sometimes with lower and sometimes with higher level of compliance. Therefore, it is reasonable to believe that the recorded events of MEMS® provided a very close proxy of the actual number of tablets taken by each woman.

### Calendar Plot

![Calendar Plot for May 1997](image1)

![Calendar Plot for June 1997](image2)

![Calendar Plot for July 1997](image3)

![Calendar Plot for August 1997](image4)

### Chronology

![Chronology Graph](image5)

*Calendar plot of one MEMS® given to a woman assigned to the 12-week daily supplementation regimen*
Information on gastro-intestinal side effects of the iron supplementation was recalled at the end of the first four weeks of the supplementation (Paper IV) and included symptoms, such as, nausea, vomiting, diarrhoea, heartburn and constipation. Side effects that occurred during the first 4 weeks were used to predict compliance during weeks 5-11 of the supplementation. As the side effects preceded compliance, the possibility of negative confounding due to reversed causality was avoided, i.e. that limited tablet intake that led to few side effects was masking the effect of side effects on compliance.

Some of the reported gastrointestinal symptoms could have been caused by pregnancy *per se*. The total reporting of the side effects could, therefore, be a mix of pregnancy-induced and iron supplementation-related symptoms. However, the aim of the analysis was a comparison between the daily and weekly groups in assessing impact of “perceived” side effects and not aimed at assessing the level of “true” side effects.

**Assessing the problem**

**Anaemia**

The results confirm the earlier findings that anaemia is highly prevalent in both non-pregnant and pregnant women in rural Bangladesh (37, 107). While the anaemia prevalence found in pregnant women (50%) was similar to that found in the National survey, there was a difference in anaemia prevalence in non-pregnant women. The prevalence as found in the present study was higher (73%) than reported in the national survey (45%) (37). A considerable regional variation in anaemia prevalence has been reported among pre-school children in nationally representative surveys in Bangladesh (37, 107). It may, thus, be that the anaemia in non-pregnant women also has a marked geographical variation and the high prevalence estimated in Mymensingh is valid. However, it can not be ruled out that some methodological problems in part also contributed to this difference. Both the National survey and the survey in Mymensingh used finger prick blood sampling. It is well-known that finger prick sampling is difficult and contribute to difficulties in surveys of anaemia prevalence (1, 118). A common problem is “milking” the finger providing a blood sample that is diluted, resulting in an overestimation of the anaemia prevalence. Another, potential source of difference between the two surveys is the techniques used to determine haemoglobin concentration. In the
Discussion

National survey HemoCue system and in the Mymensingh survey a standard laboratory technique was used. While the actual assessment of haemoglobin concentration is comparable, the technique to collect a small volume of blood is different. In HemoCue system, a disposable cuvette was filled with blood by capillary forces, which was relatively simple to use. On the contrary, in using the standard method, there was a need to collect 20 µl of blood sample with a micropipette, which was difficult and, therefore, the limitations in technique might have contributed to the difference in the prevalence estimates.

Most of the women had anaemia of either mild or moderate degree with a surprisingly low level of severe anaemia. The findings are in agreement to the National estimates (37). High prevalence of severe anaemia has been reported among pregnant women in neighbouring India (15%) (35). The question, therefore, arises why the prevalence of severe anaemia is so low among women in Bangladesh. Several factors might have contributed to this situation. Since anaemia is known to be associated with factors like malaria, hookworm infestation and HIV/AIDS (13, 52, 119-121), the presence of one or more of these factors can contribute to severe anaemia. While the prevalence of malaria, hookworm infestation and HIV/AIDS apparently is high in India (122-124), the comparative figures in Bangladesh are reportedly low (125, 126). This may partly explain the low prevalence of severe anaemia in the study.

Contribution of iron deficiency

As mentioned earlier, the proportion of anaemia that can be controlled by iron supplementation depends largely on the contribution of iron deficiency to the problem. Therefore, an indicator of iron deficiency is required that identifies who would respond to iron supplementation and, thus, receive the potential health benefits. In the present study, prevalence of iron deficiency was assessed in pregnant women using low sFt and high sTfR as indicators. No previously published information on sFt and sTfR status among pregnant women in Bangladesh was found and, thus, no comparison could be made. However, the values were within the suggested range (2, 3). Different indicators of iron deficiency represent different dimensions of the problem, and as found in the study, the prevalence shows a wide variation depending on the type of indicator used (Paper II). Since low sFt and high sTfR are indicative of reduced storage iron and tissue iron deficiency, respectively (2, 3), a combination of these indicators will be able to identify women with coexistence of both the problems. In terms of this combined indicator, prevalence of iron deficiency was
found lowest (20%) in the anaemic women. In contrast, in terms of presence of any one of these two indicators, the prevalence was found in the highest range (65%) in the same population. If the latter indicator of iron deficiency is considered, about two-thirds of anaemia in pregnant women could be controlled through the provision of iron supplements. This indicates that any statement of iron deficiency in relation to anaemia should be made carefully, specifying the indicator used.

The prevalence of low sFt increased among pregnant women in the highest haemoglobin category. This is contrary to what could be expected in a non-anaemic population and raises the question whether all women in the highest haemoglobin category really were healthy. It is conceivable that some of these women did not have had sufficient plasma volume expansion during pregnancy, leading to a pathologically high haemoglobin concentration. The same cause of insufficient plasma volume expansion might also have resulted in low sFt. It is, therefore, possible that not only anaemic women but also women with high haemoglobin can be iron deficient and in need of iron supplementation.

Supplementation

Compliance
One of the unique features in this study was the use of MEMS® for collecting data on compliance. It allowed monitoring of compliance for each woman over an extended period of time. This is the only study that has included a continuous monitoring of compliance comparing daily and weekly dose frequencies of iron supplementation. As hypothesised (127), compliance was found to be higher in weekly group. Average number of tablets taken was 52 in daily and 23 in weekly, corresponding to mean compliance of 68% and 104%, respectively. Information on compliance from other studies using MEMS is not available for weekly regimen. An iron supplementation trial among pregnant women in Tanzania recommended 120 mg of iron daily and found a compliance to be 42% after 1 months of supplementation (29). The better compliance in the Bangladesh trial despite a longer duration of supplementation period monitored may be due to a lower daily dose of iron than used in Tanzania.
Discussion

It has been hypothesised that weekly administration of iron will reduce side effects and thereby increase compliance (85). While an increased compliance was found in the study, this was not due to a less frequent occurrence of side effects. In spite of more frequent occurrence of side effects in the weekly group, mean compliance was higher in that group as compared to the daily group. It appears that side effects did not play the major role for compliance as hypothesised (1, 67). Low impact of side effects on compliance was also found in a study in Tanzania where only a third of non-compliance was explained by occurrence of side effects and, therefore, a large part of non-compliance remained unexplained (29). The findings imply that intervention to reduce side effects may not produce a major change in compliance and may, thus, not be recommended as a means to improve effectiveness of iron supplementation programmes. A number of factors apart from side effects may determine the compliance. These include appearance and quality of the supplements as well as the quality of the communication between the community health workers during distributing the supplements and pregnant women (27). Therefore, efforts to improve these factors may be a better way to increase the effectiveness of iron supplementation programmes.

While reported occurrence of side effects did not vary between different SES group the impact of side effects on compliance varied. Women with high socio-economic status maintained a similar level of compliance whether they experienced the side effects or not. However, women with low SES who reported side effects had a lower compliance than those who did not. A possibility is that women from higher SES had a better understanding of the importance of iron supplementation and were more motivated to continue the supplementation despite the felt symptoms of side effects. It has been reported that proper information on the benefits of iron supplementation as well as a preparedness of the occurrence of side effects may prevent a its negative effect on compliance.

Efficacy

Both types of evaluation of efficacy that have been reported in this study (dose frequency and regimen) required precise information on the actual number of iron tablets taken. None of the other community trials comparing effect of daily and weekly iron supplementation included a valid measure of compliance and, thus, could not evaluate the difference in haemoglobin response per iron tablet taken. In our study, the effect on haemoglobin concentration per tablet administered daily or weekly did not show any difference (Paper III). Thus, the
Discussion

finding provides no support to the “mucosal block” theory (32). However, it is in agreement with a clinical trial in which radio-labelled iron was used to assess the rate of iron absorption in humans administered daily or intermittently where only a limited difference in absorption was found (92). It was, therefore, concluded that there are no evidence that it is possible to increase the efficacy of iron supplementation during pregnancy by recommending weekly dose frequency.

In the present study, efficacy of dose regimen was predicted from a dose response curve plotting haemoglobin concentration at 12 week against number of iron tablets taken during weeks 1-11 (Figure 3). As described earlier in the thesis, there was a steep response for the first 20 tablets consumed and a maximum effect on haemoglobin concentration was observed after the intake of a total of 40 tablets. Had each of the two groups taken the prescribed number of tablets during the 12-week long supplementation period, the daily would have had a higher haemoglobin concentration than the weekly. The superimposition of the 3 dose-response curves indicates that the main determinant of the effect of iron supplementation was the number of tablets taken, not the duration of iron supplementation. This implies that while the daily regimen would produce an effect faster, the efficacy of the weekly regimen would approach to that of the daily if duration of the supplementation was longer. One of the other daily/weekly trials done in China (96) applied supervised intake and, thus, evaluated efficacy of dose regimens. Over 4 months of supervised supplementation no difference was found between the daily and weekly. The lack of difference in the Chinese study may, therefore, be explained by the longer (4 months) duration of the supplementation compared to the 3 months in the present study.

Trial effectiveness

Including the impact of compliance, a better haemoglobin response was found after 12 weeks of the daily supplementation during pregnancy as compared to the weekly. The result can be compared with “intent-to-treat” analyses in the studies done in Indonesia (95), Malawi (94) and in Pakistan (93). While the study in Pakistan also found a larger effect in the daily regimen, the other two studies did not find a difference in effect. In both the latter studies, the haemoglobin response was limited, suggesting that a lack of difference between daily and weekly may in part be due to a lack of response to the iron supplementation activity. Due to ethical reasons, none of the studies included a control group and it was difficult to judge how large the
response was. Another reason for lack of difference is a differential level of compliance between the groups, resulting in a similar level of tablet intake in the daily and weekly regimens and, therefore, a similar effect.

At 6 weeks post partum, the difference in haemoglobin concentration between the daily and weekly supplementation groups had disappeared and both the regimens produced a similar effect on haemoglobin concentration. This could not be explained by a lack of response to iron supplementation in either groups since a dose-effect relationship was shown between categories of tablet intake during pregnancy and haemoglobin concentration at 6 weeks post partum. While the haemoglobin concentration of women in the highest tablet intake category approached the concentration reported in a healthy well-nourished population (135 g/L) (129), neither the daily nor the weekly supplementation regimen had reached this concentration. It appears that the most likely explanation for lack of difference in haemoglobin concentration is that the tablet intake distribution of the daily and weekly regimens approached each other and by 6 weeks post partum tablet intake was similar in both groups, but not optimal to produce the maximum effect.

Implications for future research

Prevalence of anaemia and iron deficiency

The ultimate aim for assessing iron deficiency in this population was to predict to what extent iron deficiency anaemia could be controlled by iron supplementation. However, while there are a number of indicators used for determining iron deficiency, they have not yet been evaluated for prediction the response. It is, thus, difficult to interpret to what extent iron supplements may control anaemia in this population of pregnant women. Although iron deficiency was common, a substantial part of the anaemia observed could not be explained by iron deficiency. Therefore, it appears that there is a need to further identify what the contributing factors of maternal anaemia are. In order to design an effective intervention for the prevention and control, the aetiology of anaemia should be clearly investigated to design a package of community-specific intervention programme including iron supplementation to effectively address the problem of anaemia as well as iron deficiency.


Discussion

Compliance
Compliance has been found low, particularly in the daily regimen limits effectiveness of the programmes. The weekly iron supplementation regimen increase compliance, but it has been shown that despite higher compliance the regimen is less efficacious and effective during pregnancy. Improving compliance by reducing side effects has not been found to be an effective strategy. More focus should be given to increase motivation of the women to start taking the supplements as well as continue to do so.

Optimal dose and regimen
The maximum effect after about 40 tablets suggests that the current recommendations (4, 66) of iron supplementation during pregnancy are unnecessarily high. Further research is needed to evaluate the amount of iron required during pregnancy as well as an effective strategy to deliver this amount. Because a substantial part of the maximum response is achieved after intake of the first 20 tablets, an iron supplementation delivery strategy may be developed through which this number of tablets can be consumed as early as possible during pregnancy. The evaluation of iron requirement should not be restricted to the pregnancy period but also include post partum period.
Acknowledgements

I would like to thank my colleagues, friends and relatives who gave me their support and encouragement to complete this job. There are many names to mention. First of all I would like to express my respect and sincere thanks to all the pregnant women in Mymensingh who participated in this trial. I am grateful that they shared their limited time with the interviewers and agreed to answer a large number of questions and I would like to dedicate my thesis to them.

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Zia Hyder
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Anaemia among non-pregnant women in rural Bangladesh

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Abstract
Objective: To estimate the prevalence and severity of anaemia among non-pregnant women in rural Bangladesh and describe its social distribution.
Design: A cross-sectional study conducted in February–March 1996. Haemoglobin concentration was measured on a capillary blood sample by cyanmethaemoglobin method. The World Health Organization (WHO) classification was used to define anaemia.
Setting: Twelve randomly selected villages in Fulbaria thana of Mymensingh district, about 110 km northwest of Dhaka city in Bangladesh.
Subjects: A systematically selected sample of 179 non-pregnant apparently healthy women aged 15–45 years.
Results: Anaemia was highly prevalent (73%; 95%CI 67–79%). Most of the women had mild (52%) or moderate (20%) anaemia, but a few of them suffered from severe anaemia (1%). Ascaris was common (39%) while hookworm was not (1%). The anaemia prevalence had no statistically significant association with age, parity or Ascaris infestation (P > 0.05). Women with less than 1 year of schooling, who were landless or who reported having an economic deficit in the household had significantly higher prevalence of anaemia (P < 0.05). There was a significantly increasing trend in anaemia prevalence with decreasing socioeconomic situation (SES). However anaemia was common in all social strata.
Conclusions: Although the overall anaemia prevalence among non-pregnant rural women is high, only a few women suffer from severe anaemia. Women of all SES groups irrespective of their age and parity are affected by anaemia.

Keywords
Anaemia
Severe anaemia
Non-pregnant women
Socioeconomic
Rural
Bangladesh

Anaemia is a major public health problem. It has been estimated that 2150 million people in the world are anaemic, of whom about 50% are thought to suffer from iron deficiency anaemia1. Iron deficiency and anaemia are most prevalent among pregnant women and young children with the highest prevalence in low income countries2. Iron deficiency and anaemia during pregnancy are associated with low birth weight, preterm delivery and increased perinatal mortality3–5. Routine supplementation of iron through the primary health care system has been widely practised to reduce maternal anaemia, although the programmes have shown limited effectiveness6,7. Women of reproductive age in low income countries who are currently not pregnant are also affected by anaemia8. The most important functional consequences are fatigue and reduced productivity9. However, the recent interest in iron supplementation of non-pregnant women is mostly rationalized by its potential impact on maternal anaemia. Prepregnancy haemoglobin concentration and iron status are believed to be predisposing factors for maternal anaemia.

Several studies have found a negative association between socioeconomic situation (SES) and anaemia prevalence10–12. Women from poor households are usually found to have higher anaemia prevalence. Poor SES is known to be associated with a number of factors such as high parity, short birth interval, poor diet both in quantity and quality, lack of health and nutrition awareness, and a high rate of infectious diseases and parasitic infestations. Since SES is an important determinant of access to health care, poor people have often limited access to medical attention and preventive measures13, increasing their risk of becoming anaemic.

Bangladesh is a poor country with a present per capita gross national product of about US$280 only. According to the latest national nutrition survey14, more than 90% of the pre-school children suffer from mild to severe
protein–energy malnutrition. The prevalence of anaemia among rural pregnant and non-pregnant women was found to be 60% and 85%, respectively\textsuperscript{14}.

The government and a number of non-governmental organizations have been implementing health programmes that include prevention and control of anaemia. So far, the major intervention has been the distribution of iron/folate supplements to pregnant women. However, supplementing newly wed women has also been discussed recently.

The aim of this paper is to estimate the prevalence of anaemia and its severity among non-pregnant women in a rural area of Bangladesh, describe its socioeconomic stratification, and explore some factors commonly associated with anaemia.

**Subjects and methods**

The study was performed in 12 villages of Fulbaria thana (subdistrict) in Mymensingh district, Bangladesh, about 110 km northwest of Dhaka city. The thana was selected to represent areas covered only by government health services for women which did not have any additional antenatal health service by other providers. The study villages were selected randomly and were representative of typical rural Bangladesh. They have a high population density and are located on the plain of agricultural land. The livelihood of the population in the study area is mainly based on subsistence farming. The staple crop is rice, which is consumed with vegetables, lentils and some fish, as well as meat on rare occasions. The study was conducted in February–March 1996. This is a post-harvest period for rice and considered as the time when most of the households have the best food and economic resources. A sample of 184 women was selected based on their availability at home and consent to participate in the study. The selected women were in the age range of 15–45 years. They were all married, apparently healthy and reportedly not pregnant at the time of the study.

The study was conducted by the Research and Evaluation Division (RED) of BRAC, which is a national non-governmental organization in Bangladesh. Twelve female interviewers of BRAC/RED received a 3-day training on application of the questionnaire. They were divided into six groups, each responsible for two villages. To identify and recruit the women from a village, each team started house-to-house visits from the centre of the village and then moved in one direction until the recruitment of the required number of 15 women was completed. This sampling method has been recommended in health studies in low income countries\textsuperscript{15}.

Each woman was interviewed using a structured questionnaire. The women were provided with a plastic container to collect a sample of faeces. The next day, two laboratory technicians visited the women at their houses and picked up the stool samples. Twenty microlitre capillary blood samples were collected by fingerprick from each woman and preserved in a vial containing 5 ml of Drabkin’s solution. The samples were kept in a cold flask and then transported to Mymensingh Medical College Hospital laboratory. Haemoglobin concentration measurement was performed on the same day, using the cyanmethaemoglobin method\textsuperscript{16}. Anaemia was defined as a haemoglobin concentration <120 g l\textsuperscript{-1}, which was further categorized as mild (100–119 g l\textsuperscript{-1}), moderate (70–99 g l\textsuperscript{-1}) and severe (<70 g l\textsuperscript{-1})\textsuperscript{8}. The stool samples were diluted with sodium chloride solution and examined for the presence of hookworm ova and Ascaris by a trained laboratory technician. Of the 184 women originally selected, four refused fingerprick blood sampling and one was later found to be pregnant, leaving 179 women for final analysis.

The questionnaire included three indicators of SES: attendance in a formal school, perceived household economic status and household landholding. These indicators have been used and tested in other studies and have been found to be a valid measure of SES in rural Bangladesh\textsuperscript{17}. For school attendance, a woman who did not complete at least 1 year in a formal school was categorized as having no schooling. A woman who reported that she had attended a formal educational institution for at least 1 year was categorized as having attended school irrespective of her current ability to read or write. To obtain information on perceived economic status, a woman was asked whether she considered her household’s economic situation to have been always in deficit, occasionally in deficit, balanced or surplus in the preceding year. The four different options were explained to each woman and she was asked to categorize herself under one of the options. A household was categorized as ‘deficit’ if she answered either always deficit or occasionally deficit. The two other options were combined to a ‘non-deficit’ category. The third indicator of SES was household landholding. Due to diversity of local measurement units and the prevailing inheritance laws, it was often difficult to obtain reliable information on the total amount of landholding. To validate the information, the woman’s answer on landholding was checked with those of other adult family members and the answer that was commonly agreed upon was recorded. Households with less than 0.5 acre (0.2 ha) of land were categorized as functionally landless. This definition of landlessness has been used to target poor households for poverty-alleviation programmes in Bangladesh\textsuperscript{18}.

An SES score was constructed using a combination of the previously mentioned three SES indicators. The score ranged from 0 to 3 based on the accumulated number of positive attributes. For example, a woman who reported that she had formal schooling, was economically non-deficit and had more than 0.5 acre of land, was given an SES score of 3.

Statistical significance of association between anaemia prevalence and an individual indicator of SES was analysed.
by the chi-square test. Trend over levels of SES was evaluated by the chi-square test for trend. The association between anaemia prevalence, age, parity and *Ascaris* infestation was evaluated with multivariate logistic regression analysis controlling for SES. *P* values less than 0.05 were considered as statistically significant. The SPSS WIN 8.0 software package was used for data analysis.

The study protocol was approved by the Bangladesh Medical Research Council (BMRC) ethical review committee.

**Results**

The mean age of the women was 28 years (SD 8 years) and they had three children on average. For women who had at least one child, the average time since the last pregnancy was 33 months. *Ascaris* was prevalent (39%), while hookworm was not (1%). A majority of the study women had no formal schooling (66%) and more than half were landless (61%).

Mean haemoglobin concentration of the study women was 112 g l$^{-1}$ (SD 18 g l$^{-1}$). The anaemia prevalence was 73% (95%CI 67–79%) (Fig. 1). The prevalence of mild, moderate and severe anaemia being 52%, 20% and 1%, respectively.

In a multivariate logistic regression analysis, age, parity and *Ascaris* infestations were not significantly associated with anaemia prevalence. This was even the case when controlling for SES. Each of the three single indicators of SES, as well as the combined SES score, were found to be significantly associated with anaemia prevalence (Table 1). Thus, women with less than 1 year of schooling, who were landless or who were economically deficit showed higher prevalence of anaemia.

Using the combined SES score, a significant trend of increasing anaemia prevalence with decreasing SES was found (*P* = 0.001) (Fig. 2). Women with the lowest SES score had an anaemia prevalence which was significantly higher than any of the other three scores (*P* = 0.002). There was no significant difference between any of the other SES categories. Out of 179 women, there were only two cases of severe anaemia and both of them were in the lowest SES group.

**Discussion and conclusions**

The results in this study are based on a cluster sampling procedure. Measures were taken to ensure that the selected sample was representative of the non-pregnant population of the study villages. The villages were randomly selected. The selection of women was systematic and was done to avoid selection bias. The procedure provided an equal opportunity for women of different socioeconomic settings to be included in the study. Availability of the women around their houses and their consent to participate were the factors which determined their inclusion in the study. In the rural setting of Bangladesh, most women are present around their houses and the cultural practice is for men to be responsible for most of the outside activities including food purchase.

| Characteristics                      | n   | Anaemia prevalence (%) | 95%CI     | *P* value*
|--------------------------------------|-----|------------------------|-----------|-----------
| Education                            |     |                        |           |           |
| No formal schooling                   | 118 | 78                     | 71–85     |           |
| With formal schooling                 | 61  | 62                     | 50–74     | 0.03      |
| Land holding (decimals)              |     |                        |           |           |
| Landless                             | 109 | 79                     | 71–87     |           |
| With land 50+                        | 70  | 63                     | 52–74     | 0.02      |
| Perceived economic situation         |     |                        |           |           |
| Deficit                              | 89  | 82                     | 74–90     |           |
| Non-deficit                          | 90  | 63                     | 53–73     | 0.007     |
| SES score                            |     |                        |           |           |
| 0 (low SES)                          | 58  | 88                     | 80–96     |           |
| 1                                   | 53  | 72                     | 60–84     |           |
| 2                                   | 36  | 61                     | 45–77     |           |
| 3 (high SES)                         | 32  | 59                     | 42–76     | 0.007     |

* Chi-square test.
However, two categories of women may stay out during the day, mainly because of their occupational involvement. The first group comprises the economically weaker segment of the rural population who are known to be ultra poor and are often engaged in manual labour for survival. The second group comprises women from relatively better off families who are at least high school graduates and are engaged in professions such as teaching in a primary school located in the same village. Although they are small in number, we may have missed a few women from both these groups. The women represent different ends of the anaemia spectrum and it is likely that in part an under- or overestimation is balanced.

The study villages have features characteristic of the rest of the rural areas of Bangladesh, namely dense population, high illiteracy, agriculture-based livelihood and government-run regular health care services. Thus, there are reasons to believe that the sample villages are fairly representative of rural Bangladesh. The study was performed in the post-harvest season, when food availability is better than at other periods of the year. The seasonal variation in anaemia prevalence in Bangladesh is unknown, but, if present, would imply a possible underestimation of the average annual prevalence.

The prevalence of anaemia among non-pregnant rural women was found to be 73%, which is about two-fold higher than the global prevalence in the same population group. The prevalence is of the same magnitude as found in the latest national nutrition survey in areas around Mymensingh, indicating that anaemia is indeed a widespread public health problem in Bangladesh. Although an association between anaemia prevalence and SES was found, it was common in all SES groups. When the SES score was analysed separately for mild or moderate anaemia, no statistically significant association was found. Because of the overall high prevalence and the lack of SES clustering of mild and moderate anaemia, any future anaemia prevention and control measure should, therefore, address the whole population and not be selective in terms of SES in a rural community.

Severe anaemia has been suggested to be associated with a high risk of maternal mortality. It has been calculated that severe anaemia is associated with a 4.5-fold risk of maternal death in low income countries. In our study, however, the prevalence of severe anaemia was found to be very low. Although no national estimate is available, several studies have indicated that severe anaemia among women in Bangladesh is rare, which is supported by our study. This can be contrasted with the high prevalence found in neighbouring India where the rate of severe anaemia has been reported to be 2–27%, suggesting wide geographical differences in anaemia prevalence and factors contributing to the problem. Severe anaemia among women may have multiple causes. Since hookworm, malaria and human immunodeficiency virus (HIV) infections are each associated with high anaemia prevalence, areas where more than one of these causes are prevalent can be expected to have a higher prevalence of severe anaemia. The prevalences of hookworm infestation, malaria and HIV infections are apparently higher in India than in Bangladesh. The prevalence of hookworm infestation was low in Mymensingh and it was reported by the district health office that there were no reported cases of malaria or HIV infections.

Despite the absence of these major contributory factors, it is surprising to notice a very high prevalence of mild to moderate anaemia among the women. The diet in rural Bangladesh is largely dominated by foods of plant origin, contributing more than 86% of the total energy. Since dietary iron of plant origin has a low bioavailability, this may be considered to be one of the contributing factors, along with limited consumption of animal products. Other important factors for anaemia include chronic protein-energy deficiency, other micronutrient deficiencies and chronic infections.

The associations between anaemia and parity and age, which have been observed in studies in other low income countries, were not demonstrated in our study. This may, at least partly, be due to a limited sample size. A difference in prevalence between younger (15–24 years) and older (35–44 years) women of 25% could have been demonstrated. Thus, smaller age or parity differences could not be shown due to the size of the sample. However, a study done on healthy non-pregnant Indian Hindu and Muslim women in Fiji, and another done in Buenos Aires, did not find any such association either.

Our study points out that anaemia is highly prevalent among non-pregnant women in rural Bangladesh, but the prevalence of severe anaemia is low. Women of all SES groups are affected by anaemia irrespective of age and parity. It is important to identify the causes of anaemia so that the problem can be addressed in the most effective way.
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References

Anaemia and iron deficiency during pregnancy in rural Bangladesh

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Running head: anaemia and iron deficiency during pregnancy in Bangladesh

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Abstract

Objective: To study the prevalence of anaemia during pregnancy and its association with iron deficiency in a rural Bangladesh population.

Design: Base-line data of a community based iron supplementation trial.

Setting: Women were recruited from 50 community-based antenatal care centres in a rural area of Mymensingh, northern Bangladesh.

Subjects: Two hundred and fourteen pregnant women in second trimester participated in the study. Information of socio-economic status and reproductive history were obtained through home-visits and blood samples were collected at health centres. Haemoglobin concentration (Hb) was measured by HemoCue, serum ferritin (sFt) by radioimmunoassay and serum transferrin receptor (sTfR) by enzyme-linked immunosorbent assay methods.

Results: The prevalence of anaemia (Hb <110 g/L) was 50%, but severe anaemia (Hb <70 g/L) was absent. Iron deficiency ranged from 13 to 54%, depending on the indicator used. Low sFt (<12 µg/L) was 42% and high sTfR (>8.5 mg/L) 25%. Eighty percent of women with moderate anaemia (Hb 70-99 g/L) and half with mild anaemia (Hb 100-109 g/L) had indication (s) of iron deficiency. Four out of ten women without anaemia (Hb ≥110 g/L) also had indication (s) of iron deficiency.

Conclusions: Despite high prevalence of anaemia severe cases were absent. The prevalence of iron deficiency increased at lower Hb concentration. However, an increased prevalence was also found among women with the highest HB concentration.
Introduction

Anaemia during pregnancy is a significant public health problem. Reportedly 56% of pregnant women in low-income countries in contrast to 18% in high-income countries are affected (ACC/SCN, 2000). It is associated with a number of negative outcomes, such as pre-term delivery, low birth weight, perinatal mortality, and – for severe anaemia - with maternal death (Scholl et al, 1992; Godfrey et al, 1991; Lieberman et al, 1987; Bothwell et al, 1979). Iron deficiency has often been claimed to be the predominant cause (WHO/UNICEF/UNU, 1998; ACC/SCN, 1991). Consequently, anaemia prevention and control strategies have focused on correcting this deficiency by routine iron supplementation. Other contributing factors have often been disregarded, e.g., parasitic infestations, chronic infections and other micronutrient deficiencies.

The effectiveness of iron supplementation programmes has generally been low and recently it has been questioned whether iron deficiency is contributing as much as earlier perceived to the prevalence and severity of anaemia (WHO/UNICEF/UNU, 1998). It is generally not known how common iron deficiency is and to what extent maternal anaemia is associated with iron deficiency.

In Bangladesh, two different surveys have estimated the anaemia prevalence among pregnant women to be 50% and 59% (HKI/IPHN, 1999; Jahan & Hossain, 1998). No community-based information on prevalence of iron deficiency and other factors associated with maternal anaemia has so far been collected. The objective of this paper was to present community-based information on prevalence of anaemia and its association with iron deficiency among pregnant women in a rural area of Bangladesh.

Subjects and methods

Study area

The study was conducted May-November 1997 in rural Mymensingh, Northern Bangladesh. This plain agricultural area has a high population density, low literacy, high malnutrition and limited access to health services and is, in these regards, comparable to most
parts of the country. The diet is dominated by rice, vegetables and lentils. It is occasionally mixed with pieces of fish and less frequently with meat. Malaria is not endemic and no case of HIV has been reported in the region.

Subjects
Through its community-based antenatal care centres, BRAC, a large national private development organisation, supports the government’s antenatal care activities including provision of iron supplementation to pregnant women. The service is delivered through community based antenatal care centres (ANCC). Each ANCC covers a population of around 1,000 and is managed by a female voluntary health worker. The ANCCs operate on a monthly schedule and are usually located in a residential place.

This study used baseline information from an iron/folate supplementation trial. For the trial, 50 out of 54 ANCCs in the area were selected. In the catchment area of these ANCCs a total of 611 pregnant women with fundal height <22 cm were identified through house-to-house visits and invited to enrol in the maternity programme. At the next scheduled ANCC meeting, the first four women enrolling in the maternity programme and fulfilling the inclusion criteria for the supplementation trial (i.e., fundal height between 14 and 22 cm and no previous iron supplementation during the current pregnancy) were invited to participate. A few centres included up to 6 women. The final sample consisted of 214 pregnant women having complete baseline data on haemoglobin concentration, serum ferritin (sFt) and serum transferrin receptor (sTfR).

Data collection
Background information on age, parity, gestational age and socio-economic situation were collected from all identified pregnant women at the first household visit. Gestational age was determined by measuring fundal height. The questionnaire included three indicators of socio-economic situation: formal schooling of the woman, household landholding and perceived household economic status (BRAC, 1994). Households with less than 0.5 acre of land were categorised as functionally landless (BBS, 1998). To obtain information on perceived economic status, a woman was asked whether she considered her household’s economic situation in the preceding year to have been always in deficit, occasionally deficit,
balanced or surplus. A household was categorised as ‘deficit’ if she answered either always deficit or occasionally deficit (BRAC, 1994).
Biochemical analyses

At registration for the maternity programme a venous blood sample was collected in an untreated evacuated tube. Haemoglobin concentration (Hb) was determined in the field by use of the HemoCue® system. The system has been shown to have an accuracy and precision similar to the standard cyanmethemoglobin method (van Schenck et al, 1986). The accuracy of the HemoCue was checked daily using control cuvettes provided with the machines. A Hb <110 g/L was defined as anaemia, 100-109 g/L as mild anaemia, 70-99 g/L as moderate anaemia and <70 g/L as severe anaemia (WHO/UNICEF/UNU, 1998). Within 4 h, remaining blood was transported on ice to a central laboratory. After centrifugation, serum was taken off and frozen temporarily at -20°C for later storage in Dhaka at -70°C. Analysis of the serum samples was performed at the end of the study at Department of Nutrition, University of California Davis, USA. sFt was assessed using radioimmunoassay (Diagnostic Products, San Diego, CA). Values <12 µg/L were regarded to reflect depleted iron stores (Institute of Medicine, 1990). Soluble sTfR was assessed by an enzyme-linked immunosorbent assay (Ramco Laboratories, Houston, TX). Values >8.5 mg/L were used as indicative of functional iron deficiency (Carriaga et al, 1991).

Stool samples were collected for examination of presence of ascaris and hookworm infestations by microscopic method. The sample was diluted in sodium chloride solution and the presence of worms was evaluated by a semi-quantitative technique (Brooks et al, 1995). Stool samples were not available for four individuals with Hb <80 g/L, as they were excluded from the trial.

sFt and sTfR were not normally distributed; thus, logarithmic transformation was performed. Trend and departure from linearity were tested by Chi-square. Analyses were done by use of SPSS for Windows software package (Chicago Inc., version 7.5.1).

Informed consent was obtained. The study protocol was approved by the Ethical Committee of the Bangladesh Medical Research Council, Government of Bangladesh as well as by the Research Ethics Committee of the Medical Faculty, Umeå University, Sweden.
Results

Women participating in the maternity programme were on average 24 years (range 14 to 44 years), having their first (31%), second (22%) or third (17%), or more (30%) pregnancy. Most of them were in their second trimester based on fundal height; mean fundal height was 17.1 cm, corresponding to 21 weeks of gestation (Grover et al., 1991). Sixty five percent of the households were functionally landless, 72% perceived themselves to be economically deficit and 56% of the women had attended schools for at least a year. About 38% of the women had *Ascaris* and 1% had hookworm infestation.

Biological and social characteristics of the women identified in the community and those participating in the maternity programme differed significantly only in parity (mean 2.2 and 1.8 respectively, p=0.05). sFt concentration decreased with parity (p<0.05) and each pregnancy was associated with a mean of 4 µg/L lower sFt concentration. It can thus be expected that the women not participating had 1.6 µg/L lower sFt concentration than the women in the study.

Mean Hb was 110 g/L and 50% of the women were anaemic (Table 1). The anaemia was mild (28%) or moderate (22%) and none had severe anaemia (Table 2). The distributions of sFt and sTfR were both skewed. Median value of sFt was close to cut-off for iron deficiency while sTfR was within normal range. Almost half of the women had low sFt and one-fourth had high sTfR. The prevalence of iron deficiency ranged from 13 to 54% depending on which of the indicators or combinations of them was used.

Among the anaemic women, 65% had indication of iron deficiency (either high sTfR or low sFt) (Table 2). The proportion of women with indication of iron deficiency decreased with increasing Hb (Figure 1). This tendency was consistent for the presence of high sTfR (test for trend, p <0.01, departure from linearity p =0.97), while the presence of low sFt departed from a downward linear trend with a higher proportion of low concentrations in the highest Hb group (test for trend p <0.01, departure from linearity, p <0.05). In the subgroup with Hb 120-129 g/L, 5/29 had low sFt, while 8/20 among those with Hb ≥130 had a low value (p =0.08).
Discussion

This population of pregnant women from rural Bangladesh had a high prevalence of anaemia, but no cases of severe anaemia were identified. The participants were sampled from all identified pregnant women in an area with a low socio-economic profile, typical of rural Bangladesh. The prevalence of 50% anaemia found in our study is consistent with previously reported estimates from nation-wide surveys in Bangladesh, as is the absence of severe anaemia (HKI/IPHN, 1999; Jahan & Hossain, 1998). In other countries in the South Asian region the anaemia prevalence in pregnant women is reportedly higher. One national estimate in India is 87% with a prevalence of severe anaemia as high as 15% (ICMR, 1989). From the plain land of Nepal, a prevalence of 73% with 7% being severely anaemic has been reported (Dreyfuss et al, 2000). In Sri Lanka, 65% of pregnant women were anaemic (Atukorala et al, 1994).

Iron deficiency alone seemed not to produce severe anaemia, at least not in this setting. Hookworm infestation, malaria and HIV infections have been shown to be associated with severe anaemia (McDermott et al, 1996; Olukoya & Abidoye, 1991; Brabin et al, 1990). None of these causative factors were prevalent in the study area (Birley, 1993; Islam et al, 1999), and it is possible that this is the explanation for the lack of severe anaemia. Information on parasite infestation was lacking for 4 individuals with low haemoglobin. If all these had hookworm infestation it’s prevalence would still have been low, 3%.

Forty-two percent of the women had low sFt concentration indicating insufficient iron stores. This may both be an underestimation and overestimation of this type of iron deficiency. Inflammations, which are common in countries like Bangladesh, can elevate sFt concentration and lead to underestimation of the proportion with low iron stores (Baynes et al, 1994; Olivares et al, 1995; Lipschitz, 1990). The deficiency may also be overestimated as sFt normally decreases after the first trimester (Taylor et al, 1982).

Soluble sTfR has been suggested to be a more reliable indicator of iron deficiency since it is not affected by infection (Baynes et al, 1994; Olivares et al, 1995; Skikne et al, 1990). The prevalence of
high sTfR was 25% and thus fewer women were functionally affected by iron deficiency. A small proportion of women (13%) had both high sTfR and high sFt. This inconsistency may in part be explained by infections that falsely increased sFt values. It is also possible that it could be due to impaired utilisation of stored iron due to vitamin A deficiency (Mejia, 1992). It has been reported that 49% of the pregnant women in Bangladesh have serum retinal concentration <1.05 µmol/L with the prevalence of nightblindness being 2.7% (HKI & IPHN, 1999).

An unexpected finding is the seemingly high prevalence of low sFt among women in the highest haemoglobin concentration category. There is no obvious reason why these sFt values should erroneously be low. It is conceivable that the women in the highest Hb category, in part, are not a healthy population. Their Hb concentration may be elevated due to insufficient plasma volume expansion and their low sFt concentrations may demonstrate truly insufficient stores. We may have to expand our current thinking on iron deficiency and keep the possibility open that even women with high Hb may be a risk group for iron deficiency. The question is, would these women be benefited from iron supplementation or not?

To our knowledge there is no previously published information on sFt or sTfR among pregnant women in Bangladesh to compare our results with. Such information is also limited for the rest of the South Asian region. In a study among pregnant women in Sri Lanka mean sFt concentration was 16 µg/L and prevalence of low sFt was 57% (Atukorala et al, 1994; Goonewardene et al, 1995). In India, pregnant anaemic women had a mean sFt concentration of 20 µg/L (low 51%) (Russia et al, 1999). The corresponding figures for the non-anaemic women were 27 µg/L and 15% low sFt. Among the anaemic women as many as 86% was reported to have high sTfR concentration. On contrary, prevalence of iron deficiency was considerably lower in a presumably well-nourished group of Swedish pregnant women. In this population median sFt was 34 µg/L (low sFt 10%) and their median sTfR was 4.1 mg/L (high 11%) (Åkesson et al, 1998). Pregnant women in Bangladesh appeared to have a similar high prevalence of iron deficiency as elsewhere in the region, that is of a substantially larger magnitude than that of women in a high-income country, such as Sweden.
While the high prevalence of iron deficiency suggests that iron supplementation may be an efficacious means to improve haemoglobin concentration it is not yet clear to what extent indicators of deficiency such as low sFt and high sTfR also predict response to and, more importantly, health benefits from the supplementation. Despite an iron deficiency that warrants iron supplementation, there may be other factors that limit Hb response to iron supplementation, such as deficiencies of vitamin A, vitamin $\text{B}_{12}$ or folic acid, and chronic infection (WHO, 1972; INACG/WHO, 1989).

The Bangladesh Integrated Nutritional Program (BINP) and the succeeding National Nutritional Program (NNP) aim at providing all pregnant women in Bangladesh with iron/folic acid supplement (The World Bank, 1995). In line with the previous international iron/folate recommendation (DeMaeyer, 1989), the dose schedule recommended is two tablets per day (120 mg iron and 500 µg folic acid) starting in the second trimester of pregnancy and continued until 6 weeks postpartum. In general, such large-scale efforts to prevent and control anaemia have met with limited effectiveness (Yip, 1996). This has contributed to the discussion of alternative strategies to increase the effectiveness. One of the most recent contributions is the proposition that severe anaemia and iron deficiency should be treated as separate entities and be dealt with separately (Stoltzfus, 2001). As there was no severe anaemia in our study population, and if this is the situation in a larger part of Bangladesh, such screening does not remain an option to improve effectiveness of anaemia control programs.

For programs aimed at treating and preventing anaemia during pregnancy in Bangladesh, there appears to be no shortcuts. Before any shift from general iron/folic acid supplementation to a selective approach, indicators that predict response to supplementation need to be identified. These may, or may not, include measures and cut-off levels currently used as evidence for anaemia or iron deficiency. Furthermore, in such analyses, response to iron supplementation should not be restricted to assessments of increased haemoglobin concentration and improved iron status as these measures may not fully reflect response in terms of improved maternal health and pregnancy outcome.
Acknowledgement

We thank the women in villages Samvugonj and Dapunia, Mymensingh for their participation. We thank the BRAC field workers in Mymensingh for their support and co-operation in conducting the fieldwork, and Mr. MA Wahed of ICDDR,B, Dhaka to store the serum samples at –70°C. The study was financed jointly by BRAC, Bangladesh, The Swedish Agency for Research Collaboration with Developing Countries (SAREC); and The Swedish Society of Medicine.


References


BRAC (1994): Baseline survey report. BRAC-ICDDR,B joint research project in Matlab. Dhaka: Research and Evaluation Division, BRAC.


Table 1. Haemoglobin concentration and iron status of participating women (n=214).

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td>110 (14)</td>
<td>109</td>
<td>75-150</td>
</tr>
<tr>
<td>sFt (µg/L)</td>
<td>11.5 (3.6)</td>
<td>14</td>
<td>0-177</td>
</tr>
<tr>
<td>sTfR (mg/L)</td>
<td>6.1 (1.6)</td>
<td>6.2</td>
<td>1.7-27.7</td>
</tr>
</tbody>
</table>

Hb – Haemoglobin, sTfR – Serum transferrin receptor; sFt – Serum ferritin. 
\(^1\) Antilog of mean logarithmic value

Table 2. Prevalence of anaemia and iron deficiency in anaemic and non-anaemic participating women.

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td><strong>All women (n=214)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any type (Hb&lt;110 g/L)</td>
<td>50</td>
<td>44-58</td>
</tr>
<tr>
<td>Mild (Hb 100-109 g/L)</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Moderate (Hb 70-99 g/L)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Severe (Hb&lt;70 g/L)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTfR &gt;8.5 mg/L</td>
<td>25</td>
<td>21-31</td>
</tr>
<tr>
<td>sFt &lt;12 µg/L</td>
<td>42</td>
<td>35-49</td>
</tr>
<tr>
<td>sTfR &gt;8.5 mg/L and sFt &lt;12 µg/L</td>
<td>13</td>
<td>9-17</td>
</tr>
<tr>
<td>sTfR &gt;8.5 mg/L or sFt &lt;12 µg/L</td>
<td>54</td>
<td>47-61</td>
</tr>
<tr>
<td><strong>Anaemic women (n=107)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTfR &gt;8.5 mg/L</td>
<td>30</td>
<td>22-38</td>
</tr>
<tr>
<td>sFt &lt;12 µg/L</td>
<td>55</td>
<td>46-64</td>
</tr>
<tr>
<td>sTfR &gt;8.5 and SFt &lt;12</td>
<td>20</td>
<td>13-27</td>
</tr>
<tr>
<td>sTfR &gt;8.5 mg/L or SFt &lt;12 µg/L</td>
<td>65</td>
<td>56-74</td>
</tr>
<tr>
<td><strong>Non-anaemic women (n=107)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTfR &gt;8.5 mg/L</td>
<td>19</td>
<td>12-26</td>
</tr>
<tr>
<td>sFt &lt;12 µg/L</td>
<td>29</td>
<td>21-37</td>
</tr>
<tr>
<td>sTfR &gt;8.5 and SFt &lt;12</td>
<td>6</td>
<td>2-10</td>
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<tr>
<td>sTfR &gt;8.5 mg/L or SFt &lt;12 µg/L</td>
<td>42</td>
<td>33-51</td>
</tr>
</tbody>
</table>
Figure 1. Per cent of women with low sFt and high sTfR at different Hb concentrations.
Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: Disentangling the issues.

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Running head: Iron supplements: efficacy and trial effectiveness

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Abstract

**Background:** According to current understanding there is no increased absorption of weekly iron supplements compared to daily (no mucosal block). However, community-based trials have repeatedly shown that weekly regimen is as effective as daily. Furthermore, when differences are found these are commonly smaller than expected considering differences in amount of iron provided. The possibility of differential compliance between the regimens needs to be evaluated to explain these findings.

**Objective:** Taking compliance into account, compare efficacy and trial effectiveness of weekly and daily iron supplementation during pregnancy.

**Design:** In Bangladesh, 50 antenatal centers were randomly assigned to either 2x60 mg iron once weekly or 1x60 mg daily. Compliance was monitored by use of a pill-bottle equipped with an electronic counting device. Hemoglobin concentration was measured at baseline and after 4, 8, and 12 weeks of supplementation.

**Results:** There was no differential effect per iron tablet between weekly and daily regimens. A 12 weeks daily regimen (68% compliance) produced a small but significantly larger hemoglobin response than that of weekly (104% compliance). The first 20 tablets consumed produced most of the effect, after 40 tablets there was no further response.

**Conclusion:** There was no evidence of a mucosal block in daily regimen. Over 12 weeks, 50% of the amount of iron in a daily regimen was sufficient for maximum hemoglobin effect. The weekly regimen provided a large part of this amount, explaining the limited difference in effect. It appears that current international recommendation for iron supplementation in pregnancy is higher than necessary.

**Key words:** Iron supplementation, pregnancy, efficacy, trial effectiveness, weekly dose frequency, Bangladesh
Introduction

The controversy over the so called “mucosal block” theory in iron supplementation is not yet resolved. It was hypothesized that a first dose of iron would load the mucosa with iron and block subsequent doses from absorption (1, 2). By reducing the dose frequency to once per week, matching the mucosal turn-over in man, iron from each tablet would be better absorbed and consequently a lower amount of iron would be required. Studies in small animals support the hypothesis (2, 3) but studies in humans using radiolabeled iron have only shown a small reduction in absorption due to precedent administration of iron (4, 5). This suggests that the mucosal block effect is not pronounced in man. A daily dose frequency should therefore produce a larger hematological response due to the larger amount of iron it provides. However, while some of the community-based trials testing the mucosal block theory in practice have demonstrated a larger effect of daily supplementation (6) the difference has been less than could be expected from differences in amounts of iron between the regimens.

There are several possible explanations for the limited difference in effect between the supplementation regimens in these community-based trials. First, the population may have responded to a limited extent due to low prevalence of iron deficiency, low intake of the supplements or due to the presence of other limiting factors such as vitamin A deficiency (7) or chronic infection (8). A second type of explanation may be a differential compliance between weekly and daily supplementation resulting in ingestion of similar amounts of iron. A third, less discussed, but possible explanation is that the amount of iron provided by weekly supplementation was sufficient to produce maximum effect.

The aim of this study was to compare the response in hemoglobin concentration between daily and weekly iron supplementation and in this process differentiate between biologically and behaviorally induced differences such as compliance. Precise information on the intake of supplements was obtained by the use of a micro-chip equipped pill bottle, MEMS®, that enabled a comparison of effect per tablet consumed as well as compliance to the prescribed regimens. It was also used for an estimation of amount of iron required for maximum hemoglobin effect. This is the first study designed to evaluate the mucosal block theory in a community trial as well as to
compare both efficacy and trial effectiveness of weekly and daily iron supplementation regimens.

**Subjects and methods**

**Study population and supplementation groups**

The study was implemented in rural areas of Mymensingh thana (sub-district), Bangladesh. The area is about 110 km north of the capital Dhaka, in the rural plain areas typical of Bangladesh. Rice is the dominating food crop and is cultivated by those who have access to land. About 2/3 of the population is functionally landless and have to rely on work as manual day laborers to earn their living (9). Bangladesh has an estimated prevalence of 45% of low birth weight (10), a maternal mortality of 420/100,000 births (11), and anemia prevalence of 45% and 50% in non-pregnant and pregnant women, respectively (12), suggesting that there are serious limitations in maternal health and nutrition in the country.

Women attending antenatal care centers run by a national non-governmental organization, BRAC, participated in the study. In two areas (Samvugonj and Dapunia) 50 out of 54 BRAC community based antenatal care centers were selected. Each antenatal care center covers a population of approximately 1000 and is serviced monthly. Current services include antenatal controls, health and nutrition education and provision of iron supplements.

Each of the antenatal care centers was randomly assigned to prescribe one of the interventions: one supplement daily or two supplements each Friday. Each supplement contained the equivalent of 60 mg of iron and 250 µg of folic acid. The weekly regimen thus provided 28% of the amount of iron in the daily regimen. The tablets were produced in Norway and provided by UNICEF.

All pregnant women in the catchment area of the selected 50 antenatal care centers were identified through household visits. The pregnant women were encouraged to enroll in the services of the antenatal care center. On the day of the scheduled antenatal service the first four women who booked for service at each center and who fulfilled the primary inclusion criteria were invited to participate. The criteria were; having a fundal height between 14 and 22 cm, not
having used iron supplements during the pregnancy prior to the study start, and being apparently healthy. Informed consent to participate in the study was obtained and hemoglobin concentration in venous blood was measured in women who agreed to participate. If hemoglobin concentration was below 80 g/L the woman was excluded from study and referred for appropriate investigation and therapy including daily supplementation and monthly hemoglobin assessments.

During the course of the trial, women's hemoglobin values were assessed monthly for low hemoglobin concentration. If a single measurement fell below 75 g/L or the level of two repeated monthly examinations fell between 75 and 79 g/L, women were excluded from the study, transferred to daily supplementation and referred to the BRAC health center. Supplementation continued until 6 weeks post partum.

Methods

Compliance

Compliance (or adherence) to the recommended dose frequency was assessed by the Medication Event Monitor System, MEMS® (13). It consists of an ordinary pill-bottle equipped with a cap, which has a counting device and a small microprocessor imbedded. Each time the bottle was opened and closed, time and date were recorded. The information was later retrieved by a special reader connected to a computer. Use of this equipment permitted continuous information on compliance to be collected over an extended period of time. Bottle-opening events that occurred on the first and last day were discarded as they did not provide information on a full day. For the other days, each event was considered as one tablet taken. Compliance (%) was defined as: (mean tablets taken/prescribed number of tablets) *100.

Women assigned to the daily regimen received a MEMS® pill bottle with 100 tablets and were advised to take one tablet every day of the week. The weekly supplementation group received a MEMS® bottle with 30 tablets, and they were advised to take 2 tablets (one in the morning and one in the evening) every Friday. The supplements were distributed to the women at the antenatal clinic. The women
returned the MEMS® pill bottle after 12 weeks of supplementation, including any remaining tablets. For the rest of the study (until 6 weeks post partum), iron supplements sufficient for daily or weekly supplementation, respectively, were provided in the same pill bottles replacing the MEMS® cap with an ordinary cap.

Total number of tablets taken at week 3, 7 and 11 were collected from MEMS® and used to predict hemoglobin concentration at week 4, 8 and 12. This number of weeks rather than the number corresponding to the hemoglobin assessment was better associated with hemoglobin response. This is probably because hemoglobin concentration responded to iron supplementation with a time lag.

**Hemoglobin concentration**
Venous blood samples were collected at baseline and after 12 weeks of supplementation in an evacuated non-treated tube. Hemoglobin concentration was assessed in the field by the HemoCue® system, a portable hemoglobin-meter which uses disposable microcuvettes with good reliability and accuracy (14). The HemoCue® photometer was checked daily against its control cuvette. In addition to venous blood samples, capillary blood samples were collected for monthly monitoring of hemoglobin concentration. In pregnancy, capillary blood has on average 5 g/L lower hemoglobin concentration (15). To allow for comparison with values from venous blood samples, the capillary based hemoglobin values at 4 and 8 weeks of supplementation were adjusted by +5 g/L. As all the hemoglobin outcomes were compared across supplementation groups this correction could not introduce any bias in the comparison between weekly and daily supplementation.

**Fundal height**
Fundal height was used as an indicator of gestational age. The field assistants received a 3-day training to measure symphysis-fundal height at the Obstetric Unit of Mymensingh Medical College. Fundal height was taken at the antenatal care centers on the day of recruitment. Measurements were taken in cm with a standard plastic tape. Women were asked to empty the bladder and lie in the supine position with legs extended. Measurements were taken along the longitudinal uterine axis whereby fetal crown-rump length would be reflected. Women with fundal height 14-22 cm, which corresponded to gestational age 18-24 wk (16), were included in the study.
Information on last menstrual period (LMP) as a gestational age measure was also collected but its validity proved to be poor and information on LMP could not be used in analyses.

**Baseline characteristics**
Baseline characteristics of mid-upper-arm-circumference (MUAC), age, parity and socio-economic situation (SES) were collected on all identified pregnant women at the first household visit. MUAC was measured at the center by a TALC insertion tape to the nearest 1 mm following the standard procedure. The questionnaire included three binomial indicators of SES: formal education of the woman, household landholding and perceived household economic status. ‘With education’ was defined as ever being enrolled in a formal school, ‘with landholdings’ as household landholding \( \geq 0.5 \) acre, and ‘economically surplus’ as a self reported perception that the household had not experienced any periods of economic deficit in the preceding year. A SES score was constructed using a combination of the three indicators. The score ranged from 0 to 3 based on the accumulated number of positive attributes.

**Analytical approach and statistical methods**
The monitoring of compliance by use of MEMS® in this study enabled comparisons of efficacy and effectiveness between weekly and daily supplementation. *Efficacy* is “the extent to which a specific intervention, procedure, regimen, or service produces a beneficial result under ideal conditions”(17). In the context of this study it is interpreted as the extent to which iron tablets ingested either in a weekly or daily frequency produce a differential effect on hemoglobin. Precise information on compliance was used to relate response to amount of iron consumed. *Effectiveness* is defined as “the extent to which a specific intervention, procedure, regimen, or service, when deployed in the field, does what it is intended to do for a defined population” (17). Effectiveness is affected both by efficacy as well as by compliance. The concept of effectiveness is thus influenced by both behavioral and biological factors in contrast to efficacy that is only affected by biology (18). It is useful to address both efficacy and effectiveness as biologically and behaviorally induced limitations call for different actions. The concept of effectiveness in public health is often associated with program effectiveness (19) that includes additional programmatic factors not dealt with in this research trial. To make the distinction clear between types of effectiveness it is proposed that the measures of
effectiveness in this trial should conceptually be thought of as trial effectiveness.

Furthermore, when estimating the differences in efficacy on hemoglobin concentration between weekly and daily supplementation it was necessary to differentiate between effect per tablet taken and effect over the entire supplementation period. To differentiate between these two effects we have used the concepts dose frequency and supplementation regimen. The relation between these concepts may be expressed as:

\[
\text{Supplementation regimen (mg iron)} = \text{dose (mg iron/tablet)} \times \text{dose frequency (# tablets/week)} \times \text{duration of supplementation (weeks)}
\]

Using these concepts our study compared

a) the estimated efficacy of weekly and daily dose frequency

b) the predicted efficacy of weekly and daily supplementation regimen

c) the estimated trial effectiveness of weekly and daily supplementation regimen

The comparison of efficacy of dose frequency tested whether there was a difference in hematological response per tablet ingested in a weekly or daily dose frequency. In efficacy of supplementation regimen the difference in hematological response over a 12 weeks period was predicted as it would be if compliance had been 100%. The comparison of trial effectiveness of supplementation regimens showed the actual difference in hematological effect over a 12 weeks period including the effect of differential compliance.

The questionnaires were coded and data entered using the SPSSWIN (version 7.5.1) statistical package. Data were verified by checking for consistency and range. For data analysis SPSS-WIN 9.0 and STATA 7 statistical packages were used. The statistical methods used included Student’s t-test, analysis of variance (ANOVA), Bonferroni test for multiple comparisons, correlation analysis, 3 factor repeated measures ANOVA, multivariate-regression analysis as well as hierarchical ANOVA. Statistical significance was set at $P<0.05$. Variables were tested for normal distribution. For non normal distributions medians and percentiles were presented and comparisons were made by Mann Whitney U test statistics. Lowess (20) smoothed plots were used to visualize dose response relations.
The risk of confounding was fundamentally different between the analyses of efficacy and trial effectiveness. The efficacy analysis depended on differential compliance to produce a range of tablet intake. The number of tablets taken in the two regimens was therefore not randomized but due to individuals’ behavior and potentially associated with confounding factors. Careful analyses of potentially confounding factors were therefore essential in the efficacy analyses. In the trial effectiveness analyses the interventions, however, weekly and daily regimen, were randomized, as were any potentially confounding factors. Thus control for confounding in this type of analyses is theoretically not necessary but was done nevertheless to increase plausibility (19). All analyses were tested for the confounding effects of maternal age, fundal height, initial hemoglobin concentration, parity, and SES. For a given analysis potential confounders were identified as those variables with a \( P<0.20 \) for any linear or non-linear association with hemoglobin outcomes and either tablet ingestion (for efficacy analyses) or across the daily-weekly regimen (for effectiveness analyses). Potential confounding was accounted for by introducing as main effects and as pertinent interactions the potential confounding factors into the crucial analyses. Confounding by the measured variables was discarded as an important contribution to the main effects if the interactions of their linear or non-linear introductions did not change by more than 10% the parameters describing the dose response or describing the daily-weekly interactions with regimen.

The study was reviewed and approved by the Research Ethics Committee of the Medical Faculty at Umeå University, Sweden and the Ethical Board at Bangladesh Medical Research Council, Dhaka, Bangladesh. Informed consent was obtained verbally due to the high rate of illiteracy.

Results

Participation

Among the 209 women enrolled in the study, 140 (67%) had complete information including hemoglobin concentration after 12 weeks of supplementation and information on number of tablets taken (Figure 1). The major reason for loss to follow-up was giving
birth before the scheduled blood sample collection at 12 weeks of supplementation or technical problems with the MEMS® resulting in loss of information on compliance. The only difference between those with or without complete data was in initial hemoglobin and in fundal height at base-line (Table 1). A higher fundal height among women with incomplete data occurred to a similar extent in both daily and weekly group. More women in the weekly group with incomplete data had lower hemoglobin concentration.

**Trial effectiveness of daily and weekly regimen after 12 weeks of supplementation**

**Full sample**

The comparison of trial effectiveness of supplementation regimens includes the effect of a potential differential level of compliance to the prescribed supplementation regimens. The comparisons are those between allocation of daily and weekly regimens respectively, disregarding level of compliance. This type of analysis is often referred to as intent-to-treat analysis.

There was no significant difference in initial hemoglobin concentration between weekly and daily groups at start of supplementation (Table 2). After 4, 8 and 12 weeks of supplementation hemoglobin concentration had increased in both groups but the increment was larger in the daily regimen. However, there was no difference in attained hemoglobin concentration after 12 weeks of supplementation. Mean tablet intake was 52 and 23 in the daily and weekly regimen, respectively ($P<0.05$). This corresponded to a compliance of 68% in the daily regimen and 104% in the weekly ($P<0.05$). The analysis was repeated using a 3 factor repeated measures ANOVA (initial hemoglobin concentration, time of hemoglobin measure, and supplementation regimen) including all interactions. As expected initial hemoglobin concentration had a significant effect on the other hemoglobin measures ($P<0.05$). Accounting for this and for a clustering effect of within subject the mean attained hemoglobin concentration was significantly larger in daily (120.1 g/L) compared to weekly regimen (117.1 g/L, $P<0.05$).

The hemoglobin concentration corresponded to an initial prevalence of anemia of 50% in the daily regimen and 42% in the weekly. After 4, 8 and 12 weeks of supplementation the anemia prevalence was 30, 23 and 14% in the daily regimen and 39, 30, and 20% in the weekly.
There was no statistical difference in prevalence of anemia at any point in time between the supplementation regimens. A proportion of anemia thus persisted in both regimens.

At base-line there were no differences between women in randomization groups regarding initial hemoglobin concentration, fundal height, age, parity and SES. Thus, the trial effectiveness analysis could not be confounded by these factors.

**Subset with lower initial hemoglobin**

Women with lower initial hemoglobin (<115 g/L) concentration were selected to compare the trial effectiveness between daily and weekly regimen as these women can be expected to have a larger need for iron supplements. Such exclusion of potential non-responders may reduce the risk of not identifying a difference in effect.

Hemoglobin concentration increased faster in the daily group compared to weekly and was significantly different at week 8 of supplementation (Table 2). At 12 weeks there was both a significant difference in increment and in attained hemoglobin concentration demonstrating a larger trial effectiveness among women on the daily regimen compared to the weekly.

A similar pattern was also shown for prevalence of anemia. Both groups had an initial prevalence of about 70%. After 4 weeks the prevalence in the daily regimen had decreased to 37% compared to 55% in the weekly ($P=0.09$). The prevalence continued to decrease. After 8 weeks of supplementation it was 33% in the daily and 39% in the weekly. When 12 weeks of supplementation was completed 20% of women in the daily group were anemic compared to 34% in the weekly group ($P=0.12$).

In this subset of women, initial hemoglobin concentration, age and parity (but not SES or fundal height) were associated with hemoglobin concentration at 12 weeks. Among these, age was also associated with supplementation regimen and could thus potentially confound the analysis. In multivariate regression analysis potential confounding by age was controlled for including the bivariate interaction. Introduction of age did not change the estimated difference between the regimens (data not shown).
Antenatal care center level of analysis; subset with lower initial hemoglobin
The unit of randomization was the antenatal care center. The trial effectiveness analysis was therefore also performed using antenatal care center as the unit of analysis. If a large variation exists between the antenatal care centers a bias may have been introduced. To evaluate the difference in results the key analysis of comparing trial effectiveness between weekly and daily supplementation in the subset of women with lower initial hemoglobin concentration was also done using antenatal care center as the unit of analysis. Mean hemoglobin concentration for each antenatal care center was calculated and compared between the regimens. Mean increment in hemoglobin concentration over 12 weeks of supplementation for centers applying daily supplementation was 17.0±14.7 g/L (n=19) compared to 10.8 ±7.9 g/L (n=21) for centers using weekly supplementation. The difference was not statistically significant (P=0.10) but the power of this comparison was limited. The difference in increment (6.2 g/L) was somewhat higher than that observed in the analyses using individual women as the unit of analysis. Mean attained hemoglobin concentration at 12 weeks was higher in daily than in weekly (121.8±15.8 g/L, and 113.5±10.6 g/L respectively, P=0.056). The difference in effect between daily and weekly was also confirmed in hierarchical ANOVA analysis adjusting for the variation between antenatal care centers (P=0.054). The analyses using antenatal care center as the unit of analysis confirmed the finding at the individual level that trial effectiveness of 12 weeks of daily supplementation regimen was higher than that of weekly.

Efficacy per tablet in weekly and daily supplementation
The difference in efficacy of dose frequency evaluates the mucosal block theory. It tests the hypothesis that the same dose of iron from tablets administered daily is less efficiently absorbed and incorporated into hemoglobin than iron from tablets taken weekly. The aim of the efficacy analyses was to compare biologic differences in response to iron supplementation. The subset of women with lower initial hemoglobin concentration was used for the efficacy analyses.

Information on compliance was used to compare the efficacy per tablet between weekly and daily dose frequencies. Two criteria had to be fulfilled to enable an evaluation of difference in dose effect.
First, the comparison had to be made within a range of tablet intake where number of tablets taken and hemoglobin concentration demonstrated a dose-effect relation. Secondly, the supplementation groups had to have an overlapping distribution of number of tablets taken.

Hemoglobin concentration at 4 weeks was plotted as a function of tablets taken at week 3 and a Lowess moving average line was fitted (Figure 2). It demonstrated a steep initial response that leveled off and reached its asymptote at about 15 tablets. A subset of women, who took 15 or fewer tablets, representing the steepest segment of the curve, was selected for the comparison of efficacy between weekly and daily dose frequency. The mean tablet intake was 6.6 ± 2.0 (range 0-11, n=44) in the weekly group and 7.5± 4.9 (range 1-15, n=14) in the daily group and thus overlapped.

Using ordinary least square regression analysis a differential dose effect was tested by modeling hemoglobin concentration at 4 weeks as a function of initial hemoglobin concentration, number of tablets, supplementation group, and interaction between group and number of tablets. The regression model indicated that there was no difference in response ($P=0.54$) between weekly and daily dose frequencies and each of the first 15 tablets taken increased hemoglobin concentration by 1.2 g/L ($P<0.05$), Table 3. Iron supplements taken in a weekly dose frequency did not demonstrate an improved efficacy in increasing hemoglobin concentration and the effect per tablet was the same. Thus, there was no evidence for a mucosal block due to daily dose frequency.

The analysis was tested for possible confounding effect of maternal age, parity, fundal height, socio-economic situation and initial hemoglobin in the way described in the method section. In the subset used for this analysis initial hemoglobin and fundal height were associated with hemoglobin concentration at 4 weeks but, as they were not associated with supplement intake, they could not be confounding factors in the analysis.

**Predicted efficacy of daily and weekly regimen after 12 weeks of supplementation**

This analysis predicted the efficacy of supplementation regimens i.e. the maximum effect on hemoglobin concentration that could have been achieved over 12 weeks of supplementation if compliance had
been 100%. The subset of women with low initial hemoglobin was also selected for this analysis.

Hemoglobin concentrations at week 4, 8 and 12 were plotted as functions of tablets taken week 3, 7, and 11, respectively (Figure 3). A dose effect was demonstrated not only at week 4 of supplementation but also at weeks 8 and 12. The three lines indicating response after 4, 8 and 12 weeks of supplementation were superimposed implying that the major determinant of dose effect was the number of tablets and not duration of supplementation. The graph suggested that at 12 weeks a plateau was reached at about 40 tablets. To verify tablet intake required for maximum effect that could be achieved with iron supplements, the shape of the curve, a steep initial response that ceased at about 40 tablets, was tested. A categorical variable that divided the women into those who had taken less than 40 tablets (n=56) at week 11 and those who had taken 40 tablets or more (n=34) was constructed. Using an ordinary least square regression model the dose-effect of the first 39 tablets up to week 11 was compared to the dose-effect of 40 or more tablets. Hemoglobin concentration at 12 weeks was modeled as a function of initial hemoglobin, total number of tablets taken week 11, the 40 tablet categorical variable, as well as the interaction between number of tablets and tablet category (Table 4). An interaction was found (P<0.05) demonstrating a differential response between the first 39 tablets and higher tablet intake. A significant dose-effect was demonstrated for the first 39 tablets (0.34 g/L per tablet, P<0.05), while such a relation was not present for further tablets taken implying that there was no further hemoglobin response to taking more than 39 tablets.

The analysis was tested for potential confounding. Fundal height was not associated with hemoglobin concentration at 12 weeks. Apart from the initial hemoglobin concentration already in the model, age, parity and SES were identified as a potential confounding factors due to their association with hemoglobin concentration at 12 weeks. Of these, age was associated with tablets ingested and could act as a confounding factor. Introducing age in the model changed the estimated effect and differential response by less than 10% and age was not retained in the model.

The predicted hemoglobin response of weekly and daily supplementation over 12 weeks of supplementation, assuming 100%
compliance was estimated from the dose-effect curve (Figure 3). At 11 weeks, if women in the daily regimen had taken the 77 tablets prescribed for this period it would correspond to an attained hemoglobin concentration of 121 g/L at week 12 of supplementation. Women in the weekly regimen should have taken 22 tablets corresponding to 115 g/L of hemoglobin. The estimated increment in hemoglobin concentration would be 11.7 for the weekly regimen and 17.1 for the daily. The predicted efficacy in the weekly regimen was thus 68% of that of the daily despite providing 28% of the amount of iron. This indicated that for regimens that were 12 weeks long and had 100% compliance, a daily supplementation regimen would be more efficacious.
Discussion

**Validity**
The greater loss of women with low initial hemoglobin concentration in the weekly regimen may both lead to an under and overestimation of trial effectiveness of the weekly regimen. Underestimation as the increment in hemoglobin concentration may have been lower due to loss of more responsive women. Overestimation as attained hemoglobin concentration may be higher due to loss of women with lower concentration. However, as there was no significant difference in initial hemoglobin concentration between the regimens it is unlikely that this may have biased the results to a significant degree.

All analyses were tested for potential confounding effect of maternal age, parity, initial hemoglobin concentration, fundal height and SES. Of particular concern was the use of tablet intake as an independent measure and the potential confounding of gestational age. However, there was no association between fundal height and supplementation group or tablet intake. The procedures to control for confounding were rigorous, as described in the methods section. The results from the analyses using tablet intake as an independent variable are robust and the estimated effects were not affected by confounding factors. It is thus unlikely that the results were influenced by confounding of any practical importance.

**Efficacy and trial effectiveness of supplementation regimen**
During 12 weeks of supplementation women in the weekly regimen were prescribed to take 28% of the amount of iron prescribed to women in the daily regimen. However, in the case of full compliance, the predicted effect on increment in hemoglobin concentration in the weekly regimen was 68% of that of the daily. This was explained by lack of further hemoglobin response after intake of about 40 tablets, and consequently, almost half of the tablets in the daily regimen would not produce any effect. Based on the regression model that tested maximum effect at 40 tablets (Table 4) and the complete superimposition of the dose effect curves over time (Figure 3) it is reasonable to believe that efficacy of the weekly regimen would have approached that of daily in regimens longer than 12 weeks. The advantage of the daily regimen appears to be the shorter time required for maximum effect. It is difficult to judge the
public health importance of the observed difference in effectiveness. A more easily interpreted and probably more biologically relevant comparison is that of prevalence of anemia. Assuming that anemia of the observed degree (mild to moderate) is associated with negative pregnancy outcome, there appears to be an advantage in using a daily regimen because it resulted in fewer anemic women at end of the trial (lower initial hemoglobin subset) and treated anemia faster and thus earlier in pregnancy. Epidemiological studies have provided evidence that anemia in early pregnancy, rather than in late pregnancy, is associated with poor pregnancy outcome (21-24) suggesting that anemia should be controlled early in pregnancy.

A number of other trials have compared weekly and daily iron supplementation of pregnant women. While these trials may appear similar in design they were conceptually different and answered different research questions making comparisons difficult. Studies done in Pakistan (25), Indonesia (26) and Malawi (27), all evaluated trial effectiveness of supplementation regimen as there were no valid measures of compliance. Intent-to-treat analyses were performed without clarifying if lack of difference in trial effectiveness (26-27) was due to limitations in efficacy, compliance or a combination of those. In a trial in China (28), tablet intake was supervised and thus efficacy of supplementation regimens was evaluated.

**Efficacy of dose frequency**
A comparison of efficacy per tablet administered as weekly or daily was done in a subset of women with similar levels of tablet intake. In this subset, women in the daily group had a higher average tablet intake. The estimation of effect per tablet between the groups was thus based on different mean number of tablets taken. It is expected that the efficacy per iron tablet decreases with the number of tablets taken as absorption decreases with improved hematological status. This bias may have decreased the estimated efficacy of daily supplementation. Despite this bias in favor of a weekly dose frequency schedule efficacy was not higher in the weekly group. The conclusion that there is no improved efficacy by weekly dose frequency is in agreement with clinical trials using radiolabeled iron (4, 5). Although these trials showed a difference it was judged to be too small to be of biological importance (4).
**Amount of iron required**

Over the 12 weeks of supplementation 2400 mg of iron (40 tablets x 60 mg), was sufficient to produce maximum hemoglobin response. This is about 50% of the amount of iron recommended by WHO for the same period. Results from an iron supplementation study in pregnant women in Tanzania indicated maximum response at a similar amount of iron (29). Compliance to daily doses of 120 mg iron was monitored for one month with MEMS® and hemoglobin concentration was assessed after 12 weeks. It appears that about 50% compliance was sufficient to reach maximum effect. With our recent understanding that most of the effect occurs during the first month of supplementation, the estimated 50% compliance for maximum response can be reinterpreted as 50% of the first month’s amount of iron. This corresponds to 1800 mg of iron (50% x 30 days x 120 mg iron) and is similar to the 2400 mg estimated to be required in the Bangladeshi population.

The current recommendation by WHO is 60 mg of iron daily during 6 months of pregnancy (30) and equals to about 10800 mg iron. Based on the complete superimposition of the dose effect curves of tablets taken during the course of gestation it is reasonable to assume that only a small additional amount of iron is required to maintain maximum potential hemoglobin concentration once it has been reached. The estimated requirement of 2400 mg in Bangladesh and 1800 mg estimated in Tanzania may thus be sufficient for the full length of pregnancy. In comparison with the WHO recommendation, the amount of iron apparently required is 22 and 17% of the current recommendation, respectively.

**Program implications and future research needs**

Our results indicate that the current international recommendation on iron supplementation is inappropriately high. Considering the potential negative effects of larger than necessary amounts of iron there is an urgent need for further research establishing what is required, in particular in populations where parasite infestation aggravate the severity of iron deficiency.

We suggest that the focus should be on estimating the total amount of iron required during pregnancy and a supplementation strategy should be developed to deliver this amount. However, as the expected positive effect may occur early in pregnancy a strategy with an initial larger (daily?) dose of iron to correct an existing deficit
seems biological justifiable while a lower (weekly?) dose may be needed to maintain the achieved hemoglobin concentration. The initial dose should be set as high as possible without producing side effects that may compromise compliance. The duration of the supplementation regimen needs to be adjusted accordingly to provide the required amount of iron. Regardless of the amount of iron that finally will be recommended limited compliance is likely to be an issue and needs to be addressed in research.
References


2. Viteri FE, Liu X, Tolomei K, Martin A. True absorption and retention of supplemental iron is more efficient when iron is administered every three days rather than daily to iron-normal and iron-deficient rats. J Nutr 1995;125(1):82-91.


12. HKI. Iron deficiency anemia throughout the lifecycle in rural Bangladesh: National Vitamin A survey 1997-98. Dhaka:


Table 1. Baseline characteristics. Comparison of women with complete and incomplete measures, and comparison between women in daily and weekly regimens.

<table>
<thead>
<tr>
<th></th>
<th>Complete</th>
<th>Incomplete</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>All</td>
<td>Weekly</td>
<td>Daily</td>
<td>All</td>
<td>Weekly</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td>(n=140)</td>
<td>(n=74)</td>
<td>(n=66)</td>
<td>(n=69)</td>
<td>(n=31)</td>
<td>(n=38)</td>
</tr>
<tr>
<td>Age(^1) (y)</td>
<td>24.0</td>
<td>23.3</td>
<td>24.8</td>
<td>24.3</td>
<td>25.1</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>±5.9</td>
<td>±5.6</td>
<td>±6.2</td>
<td>±6.0</td>
<td>±8.1</td>
<td>±5.9</td>
</tr>
<tr>
<td>Parity(^2) (#)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>1 (0-3)</td>
<td>2 (0-3)</td>
<td>2 (0-3)</td>
<td>1 (0-3)</td>
</tr>
<tr>
<td>Fundal height(^1) (cm)</td>
<td>16.5</td>
<td>16.6</td>
<td>16.3</td>
<td>18.3</td>
<td>18.8(^5)</td>
<td>17.9(^6)</td>
</tr>
<tr>
<td></td>
<td>±1.8</td>
<td>±1.8</td>
<td>±1.8</td>
<td>±2.3(^4)</td>
<td>±2.3</td>
<td>±2.2</td>
</tr>
<tr>
<td>MUAC(^1) (mm)</td>
<td>223</td>
<td>223</td>
<td>223</td>
<td>227</td>
<td>229</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>±18</td>
<td>±19</td>
<td>±16</td>
<td>±22</td>
<td>±26</td>
<td>±17</td>
</tr>
<tr>
<td>Ascaris (%)</td>
<td>52/149</td>
<td>29/74</td>
<td>23/66</td>
<td>30/69</td>
<td>13/31</td>
<td>17/38</td>
</tr>
<tr>
<td></td>
<td>(37)</td>
<td>(39)</td>
<td>(35)</td>
<td>(44)</td>
<td>(42)</td>
<td>(45)</td>
</tr>
<tr>
<td>SES(^2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (0-2)</td>
<td>1 (1-2)</td>
</tr>
<tr>
<td>Hemoglobin(^1) (g/L)</td>
<td>110.6</td>
<td>112.6</td>
<td>110.4</td>
<td>107.6(^4)</td>
<td>103.9(^6)</td>
<td>110.6</td>
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<tr>
<td></td>
<td>±13.3</td>
<td>±13.9</td>
<td>±12.7</td>
<td>±14.9</td>
<td>±10.9</td>
<td>±17.3</td>
</tr>
</tbody>
</table>

\(^1\) Mean±SD, \(^2\) Median; (25\(^{th}\),75\(^{th}\) percentile), Mann-Whitney U test statistics
\(^3\) Different from all women with complete measures, \(P<0.05\), Students T-test
\(^4\) Different from all women with complete measures, \(P=0.053\), Students t-test
\(^5\) Different from women in weekly regimen with complete measures, \(P<0.05\), Bonferroni test multiple comparisons
\(^6\) Different from women in daily regimen with complete measures, \(P<0.05\), Bonferroni test multiple comparisons
Table 2. Hemoglobin concentration (Hb) at baseline, after 4, 8, and 12 wk of supplementation by supplementation groups; full sample and subset with initial Hb <115g/L.

<table>
<thead>
<tr>
<th></th>
<th>Full sample</th>
<th>Subset hemoglobin &lt;115 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weekly (n=74)</td>
<td>Daily (n=66)</td>
</tr>
<tr>
<td>Hemoglobin(^1) (g/L)</td>
<td>112.6 ± 13.9</td>
<td>110.4 ± 12.7</td>
</tr>
<tr>
<td>at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increment 4 wk</td>
<td>1.0 ± 11.3</td>
<td>4.5 ± 11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increment 8 wk</td>
<td>4.4 ± 11.6</td>
<td>7.7 ± 13.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increment 12 wk</td>
<td>10.0 ± 12.3</td>
<td>14.4 ± 14.1</td>
</tr>
<tr>
<td>attained at 12 wk</td>
<td>122.6 ± 16.1</td>
<td>124.8 ± 16.1</td>
</tr>
<tr>
<td>Tablets wk 11(^2) (#)</td>
<td>23 (17-27)</td>
<td>57 (29-75)</td>
</tr>
<tr>
<td>Compliance wk 11(^1) (%)</td>
<td>104</td>
<td>68 &lt;0.01</td>
</tr>
</tbody>
</table>

\(^1\) Mean±SD. Student’s T-tests
\(^2\) Median (25\(^{th}\)-75\(^{th}\) percentile), Mann-Whitney U test statistics
Table 3. Regression model of hemoglobin concentration (Hb) at 4 wk as a function of initial Hb, tablet intake the first 3 wk and supplementation group and the interaction between tablet intake and group; subset with initial Hb <115 g/L and women who took ≤15 tablets, n=58

<table>
<thead>
<tr>
<th>Covariate</th>
<th>β estimate</th>
<th>SEE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Hb</td>
<td>0.62</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Iron tablets</td>
<td>1.19</td>
<td>0.48</td>
<td>0.02</td>
</tr>
<tr>
<td>Group¹</td>
<td>6.80</td>
<td>5.88</td>
<td>0.25</td>
</tr>
<tr>
<td>Iron tablets * group</td>
<td>-0.47</td>
<td>0.75</td>
<td>0.54</td>
</tr>
<tr>
<td>Constant</td>
<td>97.25</td>
<td>4.26</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Dependent: Hemoglobin at 4 wk
$R^2$ 0.4

¹ Daily supplementation group coded 0, weekly coded 1

Table 4. Regression model of hemoglobin concentration at 12 wk as a function of initial hemoglobin (Hb), iron tablets taken at wk 11 and an interaction between iron tablets and tablet category; subset of women with initial Hb <115 g/L, n=90.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>β estimate</th>
<th>SEE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Hb¹</td>
<td>0.80</td>
<td>0.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Iron tablets</td>
<td>0.345</td>
<td>0.20</td>
<td>0.083</td>
</tr>
<tr>
<td>Tablet category²</td>
<td>21.37</td>
<td>9.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Iron tablet * tablet category</td>
<td>-0.470</td>
<td>0.23</td>
<td>0.047</td>
</tr>
<tr>
<td>Constant</td>
<td>108.5</td>
<td>4.44</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Dependent: Hemoglobin at 12 wk
$R^2$ 0.31

¹ value centered at mean
category codes (0-39 tablets=0, 40 or more =1)
Figure 1. Number of women randomized to intervention, lost to follow-up and analyzed by supplementation group.

50 antenatal centers randomly assigned

25 antenatal centers
Weekly regimen
105 women enrolled

25 antenatal centers
Daily regimen
104 women enrolled

Incomplete measures 31 women
- 16 delivered <12 wk of supplementation
- 1 withdrawn from participation
- 1 stillbirth
- 1 lost pill bottle
- 12 MEMS® failure

Incomplete measures 38 women
- 23 delivered <12 wk of supplementation
- 2 withdrawn from participation
- 2 hemoglobin <75 g/L
- 11 MEMS® failure

74 women for analyses
66 women for analyses
Figure 2. Hemoglobin concentration (g/L) at week 4 as a function of number of iron supplements taken at week 3, by dose frequency. Lowess moving average fitted lines. Subset with initial Hb<115 g/L.
Figure 3. Hemoglobin concentration (Hb) at week 4, 8 and 12 as functions of number of tablets taken at week 3, 7 and 11, respectively. Lowess moving average fitted line. Subset with initial Hb <115 g/L, weekly and daily regimen combined.
Do side effects reduce compliance to iron supplementation? A study of daily and weekly dose regimens in pregnancy

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\textbf{Running head:} Side-effects and compliance to iron supplementation

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Abstract

Side effects of iron supplements have been considered to lead to poor compliance. A weekly dose schedule has been suggested to produce fewer side effects than a daily regimen and therefore achieving a higher compliance. This study compared side effects and their impact on compliance among pregnant women assigned to either weekly doses of 2 x 60 mg iron (one tablet each Friday morning and evening) or daily doses of 1 x 60 mg iron.

In Bangladesh, 50 antenatal care centres were randomly assigned to prescribe either a weekly or daily supplementation regimen (86 women in each group). Side effects were assessed by recall after one month of supplementation, and used to predict compliance in the second and third months of supplementation. Compliance was monitored by use of a pill bottle equipped with an electronic counting device that recorded date and time whenever the pill bottle was opened.

Of five gastro-intestinal side effects assessed, vomiting occurred more frequently in weekly (21%) than in daily (11%, p<0.05) group. Compliance (ratio between observed and recommended tablet intake) was significantly higher in the weekly (93%) as compared to the daily supplementation regimen (61%, p < 0.05). Overall, gastro-intestinal side effects were not significantly associated with compliance. However, the presence of nausea and/or vomiting reduced compliance in both regimens – but only among women from the lower socio-economic group.

In conclusion, weekly iron supplementation in pregnancy had a higher compliance as compared to daily – in spite of its higher frequency of side effects. Our findings support the view that gastro-intestinal side effects generally have a limited influence on compliance – at least in the ranges of iron provided by the studied dose regimens. Efforts to further reduce side effects to iron supplements may not be a successful strategy for improving compliance and effectiveness of antenatal iron supplementation.

Key words: Side effect, Compliance, weekly and daily, Iron supplementation, Pregnant woman, Bangladesh

- 2 -
Background

Gastro-intestinal side effects of iron supplements have been considered to cause poor compliance and thereby resulting in ineffective supplementation programmes. A weekly supplementation schedule has been suggested to produce fewer side effects and consequently increase compliance (1). There is a great variation in occurrence of reported side effects in iron supplementation trials even with similar doses of iron. Appropriate counselling on what to expect in terms of side effects may modify the perception of side effects or counteract their negative effect on compliance (2). This study was nested into an iron supplementation trial in pregnant women in Bangladesh, where the response in haemoglobin concentration was compared between daily and weekly iron supplementation and in this process differentiating between biologically and behaviourally induced differences such as compliance (3). This paper aims at comparing side effects, compliance and impact of side effects on compliance among pregnant women assigned to either weekly doses of 2 x 60 mg iron or daily doses of 1 x 60 mg iron.

Methods

This study was performed in pregnant women in rural Mymensingh, northern Bangladesh - a plain agricultural area that has a high population density, low literacy and high malnutrition rates. Through its community-based antenatal care centres BRAC, a large national private development organisation, supports the government’s antenatal care activities including provision of iron supplements to pregnant women. The service is delivered through community based antenatal care centres (ANCC). Each ANCC covers a population of around 1,000 and is managed by a female voluntary health worker and operates on a monthly schedule.

Fifty ANCCs in the area were randomly assigned to prescribe either one iron supplement daily or two supplements each Friday (one in the morning, one in the afternoon) to participating women. Each supplement contained the equivalent of 60 mg iron and 250 µg of folic acid. Based on formative research a uniform message was developed and given at start of supplementation regarding the
Rationale for iron supplementation (good for the mother’s health), and that gastrointestinal side effects could occur (black stool, constipation, nausea and heartburn) and how to manage such side effects (taking supplement at meals). In the catchment area of these ANCCs a total of 611 pregnant women with fundal height <22 cm were identified through house-to-house visits and invited to enrol in the antenatal programme. At the next scheduled ANCC meeting, the first four to five women enrolling in the programme and fulfilling the inclusion criteria for the supplementation trial (i.e., fundal height between 14 and 22 cm and no previous iron supplementation during the current pregnancy) were invited to participate. Fieldwork was performed May 1997 to January 1998. Out of 209 participating women 172 (82%) had complete information on side effects and compliance and were included in this analysis. There was neither significant difference in background data (socio-economic status, parity), nor in allocation to randomisation groups or occurrence of side effects between those with and without complete side effect and compliance information (data not shown).

After one month of supplementation home-based interviews were performed including open-ended questions on any morbidity and gastrointestinal symptoms during the past month. Probing regarding heartburn, nausea, vomiting, diarrhoea and constipation followed this.

Medication Event Monitor System, MEMS® (Aardex, Switzerland) was used to assess compliance (or adherence) to the recommended supplementation frequency. It consists of a pill-bottle equipped with a cap, which has a counting device and a small microprocessor embedded. Time and date are recorded each time the bottle is opened. A special reader is used to download the information from the caps to the computer. Bottle opening events that occurred the first and last days of recording were disregarded, as they did not provide information of a full day and included openings at the antenatal centre. Information retrieved from week 5 to 11 of supplementation was used in this analysis. Compliance was defined as the ratio between iron tablets taken wk 5-11 over prescribed number of tablets for that time period.

Three indicators of socio-economic status were registered based on home interviews: formal schooling of the woman (never enrolled at school =0, some schooling = 1), household landholding (landholding
< 0.5 acre = 0, >= 0.5 acre = 1) and perceived household economic status (deficit household economy some period last year = 0, not deficit = 1). A SES score was constructed using the accumulation of these three indicators, ranging from 0 to 3. The score 0-1 was labelled “lower” socio-economic group and 2-3 was labelled “higher”. Reproductive histories, age, other demographic data and anthropometry were collected at start of study. Haemoglobin concentration (values not reported here) was measured by HemoCue® at baseline and monthly over the course of the trial. Women with Hb <80 g/L at baseline, with single measurements <75 g/L or two measurements 75-79 g/L during the trial were excluded from the study and provided appropriate additional investigation and treatment.

Informed consent was obtained from the participating women. The study was approved by the Ethical Committee of the Bangladesh Medical Research Council as well as by the Research Ethics Committee of the Medical Faculty, Umeå University, Sweden.

Results

In the daily as well as the weekly supplementation regimen 62% of the women reported occurrence of gastro-intestinal side effects during the first month of supplementation (heartburn, nausea, vomiting, diarrhoea or constipation, Table 1). Vomiting was reported more frequently in the weekly supplementation group, while the occurrence of other suspected side effects did not differ between the supplementation regimens.

Compliance during week 5 to 11 of the supplementation was higher in the weekly regimen (mean 93%) than in the daily regimen (mean 61%, p <0.001), Figure 1. In fact, the tablet intake in the weekly regimen ranged beyond 150% of recommended dose due to iron supplement intake by some women more than once per week. Measured from week 1 to 11 of supplementation the median intake in the daily regimen was 59 out of recommended 77 tablets (25th percentile 30, 75th 77) and 22 out of recommended 22 tablets in the weekly group (25th percentile 16, 75th 27).

Presence or absence of any side-effects wk 1 - 4 did not produce a different compliance week 5 - 11, neither in daily nor in weekly
supplementation regimen (Table 2). However, women reporting vomiting and/or nausea had lower compliance (62%) than those without these symptoms (81%) did. Compliance did not differ between socio-economic strata (76% and 79% in lower and higher strata, respectively, \( p = 0.734 \)).

Compliance week 5 to 11 differed between those who had experienced vomiting and/or nausea or not during the initial month of supplementation (Table 3). However, only women in the lower socio-economic stratum had a lower compliance when reporting side effects. In the higher socio-economic group the side effects vomiting and nausea had no influence on compliance. Baseline haemoglobin concentration, BMI at start of study, age or parity did not influence compliance week 5-11 (data not shown).

**Discussion**

We have shown that compliance to iron supplementation in pregnancy was higher in a weekly as compared to a daily supplementation regimen, despite that women in the weekly regimen did not have less but slightly more gastro-intestinal side effects. Side effects during the first month of supplementation did not have any major effect on compliance, although nausea and/or vomiting were followed by reduced compliance in women of a lower socio-economic stratum.

The design of the study included careful recall of side effects during the first month of supplementation, followed by as valid measurements of compliance as possible during the subsequent two months of supplementation – by an electronic counting device in the pill bottle cap (4). Women were unaware of the microprocessor in the cap, which had an appearance of an ordinary pill bottle cap. The prospectively collected data make causal interpretations of side effects and subsequent compliance plausible. The perceptions of side effects and the level of compliance to a certain supplementation regimen may vary between geographical settings, culture and socio-economic group. Some of the gastro-intestinal symptoms associated with use of iron supplements are also frequently caused by the pregnancy itself, e.g. nausea, vomiting and, especially later in pregnancy, heartburn and constipation. Despite probing of symptoms due to use of supplements the total frequency of reported gastro-
intestinal symptoms may be a mix of pregnancy-induced and iron supplement-induced symptoms. However, this is not confounding the analysis since the focus is on the comparison between supplementation regimens, and not on the assessment of the absolute level of side effects. Side effects are dose-dependent (5), and current data suggest that a dose of 30-60 mg per day may have a low or acceptable level of gastro-intestinal side effects (6). The weekly dose of 60 mg iron Friday morning and evening that was used in this study resulted in a slightly elevated frequency of reported side effects in comparison with a daily dose of 60 mg. This may be due to a larger daily amount of iron ingested. The occurrence of side effects has not been reported to vary with type of iron compound used (7), but with type of iron preparation. The so-called sustained-release preparations produce fewer side effects but carry a higher cost (8).

Gastro-intestinal side effects have often been blamed to be the main reason for limited compliance (9, 10), while others have not shared that view (11). Our findings support the view that gastro-intestinal side effects have a limited influence on compliance – at least in the ranges of iron provided by the studied dose regimens.

We did not find any variation in frequency of side effects between socio-economic groups, but a differential influence of side effects on compliance in socio-economic groups. The ability to understand and interpret side effects may counteract any negative effects on compliance (2). This may hypothetically vary with socio-economic group and explain our finding.

Actual information on occurrence of side effects in iron supplementation programs and their impact on compliance are limited. A study in Tanzania showed that women experiencing side effects reduced their iron supplement intake by one-third, but a large part of the non-compliance remained unexplained when the impact of side effects had been taken into account (12).

A lot of emphasis has been given to the reduction of side effects, for example by alternative iron preparations (12, 13) or by intermittent dose regimens, e.g. weekly dose schedules (1). The seemingly rational thought to reduce side effects and thereby increasing compliance and effectiveness of the supplementation may not be so rewarding. Efforts to improve compliance should rather be focused
on appropriate and acceptable supplementation regimens and motivation of the recipients of the supplements.

**Acknowledgement**

This study was funded by BRAC, Bangladesh, SAREC (The Swedish Agency for Research Collaboration with Developing Countries) and the Swedish Society of Medicine. UNICEF provided iron supplements free of charge, and Aardex, Switzerland, provided MEMS® at reduced cost.
References


Table 1. Frequency of reported symptoms (possible side effects) after one month of antenatal iron supplementation in daily (n = 86) and weekly (n = 86) supplementation groups

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Reported frequency (%) in daily supplementation</th>
<th>Reported frequency (%) in weekly supplementation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heartburn</td>
<td>11.5</td>
<td>13.6</td>
<td>0.658</td>
</tr>
<tr>
<td>Nausea</td>
<td>14.4</td>
<td>17.5</td>
<td>0.551</td>
</tr>
<tr>
<td>Vomiting</td>
<td>9.6</td>
<td>21.4</td>
<td>0.019</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>10.6</td>
<td>14.6</td>
<td>0.389</td>
</tr>
<tr>
<td>Constipation</td>
<td>59.6</td>
<td>61.2</td>
<td>0.821</td>
</tr>
<tr>
<td>Any of the five</td>
<td>61.5</td>
<td>62.1</td>
<td>0.930</td>
</tr>
</tbody>
</table>

Table 2. Mean compliance (ratio between observed tablet intake and recommended tablet intake week 5-11 of supplementation, %) to antenatal iron supplementation in daily and weekly supplementation groups with and without reported side effects week 1-4.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Side effects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>No</td>
</tr>
<tr>
<td>Daily supplements (n = 86)</td>
<td>61.1</td>
<td>67.1</td>
</tr>
<tr>
<td>Weekly supplements (n = 86)</td>
<td>92.7</td>
<td>104.5</td>
</tr>
</tbody>
</table>
Table 3. Compliance (estimated mean %) to iron supplementation week 5-11 of supplementation analysed in relation to reported vomiting or nausea during the first month of the supplementation period and socio-economic (SES) group. Adjustment is done for supplementation regimen (daily or weekly) and parity. Analysis of variance, $F = 4.6, p = 0.034 (n = 172)$.

<table>
<thead>
<tr>
<th>SES group</th>
<th>Vomiting and/or nausea</th>
<th>Estimated mean (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>No</td>
<td>81.9</td>
<td>73.0 - 90.9</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>47.9</td>
<td>30.5 – 65.3</td>
</tr>
<tr>
<td>High</td>
<td>No</td>
<td>80.5</td>
<td>68.3 – 92.7</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>82.5</td>
<td>55.6 - 109.3</td>
</tr>
</tbody>
</table>
Figure 1. Compliance (ratio between observed number of tablets and recommended number of tablets) to iron supplementation wk 5-11 of supplementation is shown for daily and weekly supplementation regimens (cumulative %). Median compliance in daily group (cumulative per cent = 50) is 65%, in weekly group 93%.
Impact of daily and weekly iron supplementation to women in pregnancy and puerperium on haemoglobin and iron status six weeks post partum: results from a community-based study in Bangladesh.

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Running head: Impact of iron supplementation in the post partum period.

Sponsorship: BRAC, Bangladesh, SAREC (The Swedish Agency for Research Collaboration with Developing Countries) and the Swedish Society of Medicine. UNICEF provided iron supplements free of charge and MEMS® was provided at reduced cost by Aardex, Switzerland

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Abstract

Three months of weekly iron supplementation during pregnancy has been found less efficacious and effective than daily in improving haemoglobin concentration and iron status. However, it has been suggested that the effect of weekly will approach that of daily if the duration of supplementation is longer. This study aimed at comparing trial effectiveness at 6 weeks post partum of daily and weekly iron supplementation during pregnancy and puerperium. In rural Bangladesh, 50 antenatal centres were assigned randomly to 1 x 60-mg iron daily or 2 x 60-mg once weekly. Data on women (daily, n = 67; weekly, n = 79) with information on haemoglobin, serum ferritin (sFt) and serum transferrin receptors (sTfR) at baseline and at 6 weeks post partum and iron tablet intake weeks 1-4 are reported. The intake of tablets was monitored by pill-bottles equipped with electronic counting devices. At 6 wk post partum, attained haemoglobin, sFt and sTfR did not differ between groups, but a larger increment of sFt was found in daily ($p=0.03$). There was a dose dependent relationship of iron supplementation during pregnancy on haemoglobin concentration still at 6 weeks post partum. Approximately one quarter of participating women in both groups were anaemic at 6 wk post partum. In a subset of women with initial haemoglobin $<$115 g/L, both the increment and the attained sFt was higher in daily group ($p=0.002$ and 0.01, respectively). Thus, we conclude that although there was no difference in trial effectiveness between the regimens in terms of haemoglobin concentration, the daily was more effective in improving iron stores at 6 weeks post partum.

Keywords: iron supplementation, pregnancy, postpartum, trial effectiveness, weekly dose frequency, serum ferritin, serum transferrin receptors, Bangladesh
Introduction

Although anaemia has the highest prevalence during pregnancy, it is common in women during other stages of the reproductive cycle including post partum period (1). Post partum is an important stage of the reproductive cycle determining women’s subsequent health and nutrition and forming the platform for the next pregnancy. Anaemia may frequently be caused by deficiencies of iron and other nutrients (2, 3). As shown elsewhere, women in low-income societies commonly suffer from nutritional deficiencies before and after pregnancies (4). Consequently, the high prevalence of post partum anaemia may have long-term implications particularly in societies where the rates of repeated and closely spaced pregnancies are common (5). Therefore, prevention and control of post partum anaemia is an important issue within the broader context of improving maternal and child health. Anaemia prevention and control strategies are commonly directed towards pregnant women in order to address adverse outcome during pregnancy (6). The current recommendation is to provide 60 mg iron and 400 µg folic acid daily during pregnancy and puerperium (7). But, despite many efforts, these programs have in general met with limited success (8). To increase the program effectiveness, an intermittent dose frequency of iron supplementation was suggested (9), resulting in a number of field trials to test the efficacy and effectiveness of weekly regimens. These trials compared the effects of iron supplementation between daily and weekly regimens in different population groups including pregnant women (10-12). However, most of these trials reported contradicting findings regarding the equivalence or superiority between regimens. A meta analysis comparing the two dose frequencies concluded that although a daily regimen was relatively better, both were efficacious to prevent a fall in haemoglobin concentration during pregnancy (13). None of the studies, however, compared the effect of daily and weekly pre-natal regimens of iron supplementation on outcomes in the post partum period.
In addition to haemoglobin concentration and iron status during pregnancy, two factors are major determinants for haematological status post partum; amount of bleeding during delivery and compliance to iron supplementation post partum. Recently, a community-based trial was conducted comparing the effect of iron supplementation provided in daily and weekly frequencies (14). The study indicated that the efficacy of a 12-week daily regimen during pregnancy was higher as compared to weekly, but also suggested that in a longer supplementation period the effect of a weekly regimen could approach that of daily. The aim of this paper was to investigate whether the effect of weekly regimen would approach that of daily if the supplementation was sustained until 6 wk post partum or, if the difference observed between the regimens would increase as a result of increased need of iron.

Subjects and Methods

Study area
The study area is located in Mymensingh district in northern Bangladesh. The area is representative of the plain land in the country, that is, having a high population density, high rates of illiteracy and malnutrition and limited access to health services. To assist in the implementation of the government health policies, BRAC, the largest national non-governmental organization in Bangladesh, distributes iron tablets to the pregnant women through its community-based antenatal care centres. BRAC antenatal care centres are available in 30% of the country and in Mymensingh district an estimated 60% of the total area is covered. Each centre covers about 1000 population and is managed by a female Community Health Worker. The study was conducted between May 1997 and October 1998.

Iron folate supplementation
Fifty antenatal care centres were randomly assigned to prescribe either daily (current program) or weekly dosages of iron supplementation. Women who were prescribed to daily supplementation were advised to take one iron tablet every evening and women in the weekly group were advised 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 sulphate and 250 µg folic acid. The supplements were of red colour, 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 post partum.

**Subjects**
Through household visits, all reproductive aged women serviced by 50 ANCCs were screened for pregnancy. Information on socio-economic situation (SES) and reproductive history was collected on identified pregnant women having a fundal height <22 cm that is judged to correspond to gestational age <24 weeks (15) and were encouraged to receive services from their respective centre. 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. Two hundred and nine pregnant women were enrolled in the iron supplementation trial, 104 in daily and 105 in weekly groups. The details of the enrolment procedure have been described elsewhere (14). 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 as described in the same study as already indicated (14). A SES score was constructed, which ranged from 3 (highest SES) to 0 (lowest SES). Fundal height was used to indicate the length of gestation and all women with a fundal height from 14-22 cm, corresponding to a gestational age 18-24 weeks (15), were considered eligible for participation in the study. Intake of iron tablet was monitored by Medication Event Monitoring System (MEMS®), a specialised pill bottle with an electronic counting device embedded in the cap. In the analysis in this paper, tablet intake during the first 4 weeks was used as an indicator of total tablet intake. Further, tablet intake was also divided into three equally sized categories representing low, medium
and high intake. Haemoglobin concentration was assessed on venous blood by HemoCue® system. Anaemia was defined according to WHO, haemoglobin concentration <110 g/L in pregnant women and <120 g/L in post partum women (6).

Venous blood was collected in an untreated evacuated tube at baseline and at 6 weeks post partum and was transported on ice within 4 h to the field laboratory. The blood was centrifuged at the field laboratory at room temperature for 5 minutes and the serum was taken off and frozen at -20°C. The frozen samples were transported on dry ice to the ICDDR,B (ICDDR,B: Centre for Health and Population Research) laboratory in Dhaka and stored at -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. The laboratory assessments were done on two parameters of iron status including serum ferritin (sFt) and serum transferrin receptors (sTfR). sFt was assessed using radio immunoassay (RIA) (Diagnostic Products, San Diego, CA). sFt values <12 µg/L were considered ‘low’, reflecting depleted iron stores (16). sTfR were assessed by an enzyme-linked immunosorbent assay (ELISA) (Ramco Laboratories, Houston, TX). sTfR values >8.5 mg/L were considered ‘high’, indicative of tissue iron deficiency (17). For the purpose of our study iron deficiency has been defined as having low sFt and/or high sTfR.

The protocol was approved by the Ethical Committee of the Bangladesh Medical Research Council (BMRC), Bangladesh and Research Ethics Committee of the Medical Faculty, Umeå University, Sweden.

**Data analysis**

The distribution of sFt and sTfR were skewed and therefore normalised by a natural logarithmic transformation before analysis. Medians were used as a measure of their central tendency, while means were used in case of haemoglobin concentration and other variables with normal distribution. Original values were used in case of haemoglobin concentration and transformed values were used in case of sFt.
and sTfR to perform all statistical tests. Differences in baseline characteristics between groups were tested by Student’s t-test. The difference in haemoglobin concentration, sFt and sTfR between daily and weekly supplementation groups was also tested by Student’s t test. The initial sFt was controlled in a linear regression analysis to examine if there was any significant difference in the attained level at 6 weeks post partum. Multiple regression analysis was used to test the effect of iron supplementation during pregnancy on haemoglobin concentration at 6 weeks postpartum. The difference between categorical variables was tested by chi-square test. Statistical significance was defined as $p<0.05$. Data were analysed by use of SPSS for WINDOWS, Release 7.5.1 (SPSS Inc, Chicago).

**Results**

Of the 209 pregnant women who were enrolled in the iron supplementation trial, 63 were lost to follow-up before 6 weeks post partum period. The reasons of lost to follow up included errors in MEMS® that resulted in loss of iron tablet intake data (n=29), refusal of the final blood sampling (n=31) and withdrawal from the trial at any time during pregnancy (n=3). Data have been 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 post partum (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 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 the women included in the analyses there was no difference in baseline characteristics except for sFt that was higher in the weekly group ($p=0.06$).

To evaluate the trial effectiveness of iron supplementation after childbirth, the change and attained values of haemoglobin concentration, sFt and sTfR at 6 weeks post partum were compared between daily and weekly supplementation...
regimens, respectively. 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 post partum (Table 3). However, a larger increment of sFt was observed among women in the daily regimen. Furthermore, there was no significant difference between the daily and weekly regimen in the prevalence of anaemia (27% vs. 22%), low sFt (8% vs. 9%) and high sTfR (24% vs. 16%), respectively at 6 weeks post partum.

Women with lower initial haemoglobin concentration (<115 g/L) were selected to evaluate trial effectiveness in a group of women with potentially higher response. In this sub-set of women, similar to the findings analysing 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 as compared to weekly at 6 weeks post partum. 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 subset of women with initial haemoglobin <115 g/L the effect of tablet intake on haemoglobin concentration at 6 wk post partum was tested in multiple regression analyses controlling for initial haemoglobin concentration and SES. The number of tablets taken after 4 wk of supplementation in pregnancy was predictive of haemoglobin concentration at 6 wk post partum ($p=0.06$).

**Discussion**

We have shown that the trial effectiveness of iron supplementation during pregnancy and puerperium did not differ between women assigned to either daily or weekly regimens evaluated as haemoglobin concentration at 6 weeks post partum. However, the daily regimen was more effective in increasing and sustaining iron status after childbirth.
A larger number of women in daily supplementation group were lost to follow up because they refused blood sampling at 6 weeks post partum (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 concentration 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 lead to an underestimation of sFt at 6 wk post partum in this group. On the other hand, it may have overestimated the increment as women with lower sFt can be expected to have a larger response.

The major focus of our 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 were randomised and, therefore, the potential confounding factors were expected to be randomly allocated between groups. In our study, the initial sFt is the only baseline characteristic that was not equally distributed between groups. This might 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 post partum. 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 vs. weekly regimens**

At 6 weeks post partum, 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 sub-set of women with lower haemoglobin concentration (<115 g/L). Several possibilities may be discussed to explain the finding of ‘no difference’ in the effect at 6 weeks post partum.
One possibility could have been that neither women in the daily nor in the weekly group responded to iron supplementation. The lack of response could be attributable to reasons including limited compliance or presence of other factors that limit the response such as vitamin A deficiency (3, 18). The observed increase in haemoglobin concentration could, in such case, be due to reversal of haemodilution that occur at late pregnancy (19) or positive iron balance after childbirth (20). However, in our study, this possibility was ruled out by showing that number of iron tablets was predictive of haemoglobin concentration at 6 weeks post partum and, thus, a lack of response may not explain the lack of difference in effect between the daily and weekly dosages of iron supplementation.

The next possibility is that the total number of tablets consumed in the two supplementation regimens were similar and, therefore, a similar effect was produced. Information on tablet 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 (14) as many of the women in the daily group took fewer tablets and weekly women took more tablets than prescribed. It is, thus, conceivable that differential compliance between the groups lead to a similar number of tablets consumed at 6 weeks post partum.

The third possibility is that the number of supplements provided by weekly regimen was sufficient to produce maximum response. This was the explanation for smaller than expected difference in response between daily and weekly supplementation evaluated after three months of supplementation during pregnancy (14), but it is not possible to conclude whether this was the case at 6 wk post partum. The fact that a mean haemoglobin concentration for healthy well nourished non-pregnant women has been reported to be 135 g/L (21), suggest that neither women in the daily nor the weekly regimen fully reached what can be considered as a normal haemoglobin concentration. Still they may have
reached the maximally achievable effect by iron supplementation in this setting. However, women in the category of largest tablet intake had a haemoglobin concentration of 134 g/L at 6 wk post partum after adjusting for effect of initial haemoglobin concentration and SES suggesting that neither the average daily nor weekly supplementation reached the response that could be achieved by iron supplements in this setting.

Thus the most likely explanation for a lack of difference in haemoglobin response at 6 wk post partum between daily and weekly iron supplementation is overlapping distributions by the two regimens. It should be noted that neither regimen fully normalised the post partum haemoglobin concentration; most likely reflecting a sub-optimal total iron tablet intake as well as the multiple causes of anaemia in pregnancy and puerperium.

**Acknowledgement**

This study was funded by BRAC, Bangladesh, SAREC (The Swedish Agency for Research Collaboration with Developing Countries) and the Swedish Society of Medicine. UNICEF provided iron supplements free of charge, and Aardex, Switzerland, provided MEMS® at reduced cost.
References


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

<table>
<thead>
<tr>
<th>Reason</th>
<th>Iron Supplementation dose</th>
<th>Daily</th>
<th>Weekly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled in the trial</td>
<td></td>
<td>104</td>
<td>105</td>
</tr>
<tr>
<td>No compliance data from wk 1-4 due to errors in MEMS®</td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Withdrew from the trial</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Refused blood sampling at 6 wk post partum</td>
<td></td>
<td>21*</td>
<td>10</td>
</tr>
<tr>
<td>Had complete information at 6 wk post partum</td>
<td></td>
<td>67</td>
<td>79</td>
</tr>
<tr>
<td>With baseline Hb &lt;115 g/L</td>
<td></td>
<td>46</td>
<td>47</td>
</tr>
</tbody>
</table>

*Difference between daily and weekly is significant, P<0.05; Hb = Haemoglobin concentration
Table 2. Baseline characteristics of the study women and women lost to follow up.

<table>
<thead>
<tr>
<th></th>
<th>With complete information</th>
<th>Lost to follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily n=67</td>
<td>Weekly n=79</td>
</tr>
<tr>
<td>Age, y&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24.8 ±6.0</td>
<td>24.0 ±5.7</td>
</tr>
<tr>
<td>Parity, #&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.5 ±1.3</td>
<td>1.4 ±1.3</td>
</tr>
<tr>
<td>Fundal height, cm&lt;sup&gt;1&lt;/sup&gt;</td>
<td>17.3 ±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2 ±2.0</td>
</tr>
<tr>
<td>SES score&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.3 ±0.6</td>
<td>1.1 ±0.8</td>
</tr>
<tr>
<td>Hb, g/L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>110 ±15</td>
<td>112 ±13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>sFt, µg/L&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12.4 ±2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.3 ±14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>sTfR, mg/L&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6.2 ±5.9</td>
<td>6.0 ±6.3</td>
</tr>
</tbody>
</table>

SES=Socio-economic status; sFt=Serum ferritin; sTfR=Serum transferrin receptor; 1=mean±sd; 2=median; Corresponding superscript letters indicate significant difference, a and b, p<0.05; c, p=0.06
Table 3. Haemoglobin concentration and iron status at baseline and 6th week post partum by dose frequency of iron supplementation; full sample and a sub-set with initial Hb <115 g/L.

<table>
<thead>
<tr>
<th>Iron supplementation frequency</th>
<th>Full sample</th>
<th>Sub-set, Hb &lt;115 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily n=67</td>
<td>Weekly n=79 P Daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weekly n=46 P</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>110</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>±15</td>
<td>±13</td>
</tr>
<tr>
<td>Change 6 wk PP</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>±12</td>
<td>±18</td>
</tr>
<tr>
<td>Attained 6 wk PP</td>
<td>128</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>±18</td>
<td>±18</td>
</tr>
<tr>
<td>SFt, µg/L</td>
<td>12.4</td>
<td>20.3</td>
</tr>
<tr>
<td>At baseline</td>
<td>41.7</td>
<td>32.0</td>
</tr>
<tr>
<td>Change 6 wk PP</td>
<td>57.6</td>
<td>57.3</td>
</tr>
<tr>
<td>Attained 6 wk PP</td>
<td>-0.5</td>
<td>-0.1</td>
</tr>
<tr>
<td>sTfR, mg/L</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>At baseline</td>
<td>6.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Change 6 wk PP</td>
<td>-0.5</td>
<td>-0.1</td>
</tr>
<tr>
<td>Attained 6 wk PP</td>
<td>4.9</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Hb=Haemoglobin concentration; SFt=Serum ferritin; sTfR=Serum transferrin receptor; 1=Mean±SD; 2=Median