Effectiveness of the Daily and Weekly Doses of Iron Supplementation in Pregnancy and Puerperium on Haemoglobin and Iron Status at 6 Weeks Postpartum

S.M. Ziauddin Hyder, MBBS, DAND, MPS (FNP)[1]
Lars-Åke Persson, MD, PhD[2]
AMR Chowdhury, PhD[3]
B Lönnerdal, PhD[4]
Eva-Charlotte Ekström, PhD[5]

[1] BRAC, Dhaka, Bangladesh and Umeå University, Sweden
[2] ICDDR,Bangladesh, and Umeå University, Sweden
[3] BRAC, Dhaka, Bangladesh
[4] Department of Nutrition, UC Davis, USA
[5] Umeå University, Sweden

EFFECTIVENESS OF THE DAILY AND WEEKLY DOSES OF IRON SUPPLEMENTATION IN PREGNANCY AND PUERPERIUM ON HAEMOGLOBIN AND IRON STATUS AT 6 WEEKS POSTPARTUM

S.M. Ziauddin Hyder[1, 2], Lars-Åke Persson[2, 3], AMR Chowdhury[2], Bo Lönnerdal, Eva-Charlotte Ekström[2, 3]

[1]BRAC, 75 Mohakhali, Dhaka 1212, Bangladesh. [2]Epidemiology, Department of Public Health and Clinical Medicine, Umeå University, Umeå S-90186, Sweden. [3]ICDDR,B: Centre for Health and Population Research, Dhaka 1212, Bangladesh. UC-Davis, CA 95616, USA.

Running head: Effectiveness of iron supplementation in pregnancy and puerperium

Abstract

Objective: Describe the effectiveness of continuous daily and weekly doses of iron supplementation during pregnancy and puerperium on haemoglobin and iron status at 6 weeks postpartum.

Design: An iron supplementation trial conducted at antenatal care centre (ANCC) assigned randomly either to the daily (1x60-mg) or weekly (2x60-mg) doses of iron supplementation.

Setting: Fifty community-based ANCCs in a rural area of Bangladesh.

Subjects: In the trial, 209 pregnant women during second trimester were recruited. Fifty-one were lost to follow-up and the final sample size consisted of 158 women (daily n=72 and weekly n=86).

Intervention: Each woman in the daily group received 1 tablet (60 mg Fe) per day and in the weekly group 2 tablets (120 mg Fe) per week. Intake of iron tablets was monitored by the MEMS® during 11 weeks in pregnancy. Haemoglobin was assessed on venous blood by HemoCue®, serum concentrations of ferritin by radio immunoassay and serum transferrin receptors by immunoassay double sandwich method.

Results: At 6 weeks postpartum, haemoglobin and iron status did not differ between the two supplementation groups. However, an overall improvement in

haemoglobin and iron status was observed compared to the baseline status. Women who took ≥40 tablets in 11 weeks had higher haemoglobin (P=0.05) and SFt (P=0.04) and lower sTfR (P=0.32) at 6 weeks postpartum.

Conclusions: A lack of difference in response was observed between the daily and weekly doses of iron supplementation. Higher number of iron tablet intake during pregnancy and puerperium was associated with better haemoglobin and iron status at 6 weeks postpartum.

Sponsorship: SIDA/SAREC, Sweden and BRAC, Bangladesh.

Descriptors: Anaemia, iron deficiency, daily weekly supplementation, postpartum, Bangladesh

Introduction

Despite many public health interventions, anaemia remains highly prevalent in all age and sex groups (WHO, UNICEF & UNU, 1998). The highest prevalence is observed in women of reproductive age (WHO, 1992). Major proportion of anaemia is caused by iron deficiency (Bothwell & Charlton, 1984) and is associated with a number of adverse outcomes. In addition to its adverse consequences on immune system, iron deficiency anaemia is associated with fatigue, reduced working capacity and ability to earn income in non-pregnant women (Untoro et al., 1998; Dallman, 1982; Li et al., 1994; Levin, 1986; Dallman, 1987). Premature delivery, low birth weight and increased perinatal mortality are associated with anaemia in pregnant women (Agarwal et al., 1991; Bothwell et al., 1979; Dreyfuss, 1998; Klebanoff et al., 1991; Murphy et al., 1986). Because of the assumption that iron supplementation would reverse these adverse outcomes, the major intervention for its prevention and control has been the provision of iron tablets during pregnancy (De Maeyer, 1989; Stoltzfus & Dreyfuss, 1998). However, little is known about the long-term effect of such interventions on haemoglobin and iron stores.

In recent years, maintaining adequate iron nutrition in non-pregnant women to prevent maternal anaemia has gained increasing interest. Among other reasons, pre-pregnancy haemoglobin and iron status have been suggested to be strong predictors of haemoglobin concentration during pregnancy (Kaufer & Casanueva, 1990). Also, doubts exist about the benefit of iron supplementation in pregnant women to reverse the adverse outcomes even when antenatal iron

supplementation programmes are in place (Cook & Reddy, 1995; Sloan et al., 1992; Viteri, 1994; Yip 1996). Therefore, extending the benefits of the iron supplementation after childbirth is important not only to sustain women's good health and nutrition, but also to reduce the adverse outcomes in subsequent pregnancies. This is particularly important in countries where malnutrition is widespread and birth-intervals are shorter.

Many studies have documented the benefits of iron supplementation in pregnancy to increase haemoglobin concentration and iron status (For example: Charoenlap et al., 1988; Chanarin et al., 1971; Kuizon et al., 1983; Menendez et al., 1994; Milman et al., 1994; Preziosi et al., 1997; Suharno et al., 1993). In recent years a new intervention strategy has been tested comparing the effectiveness of the daily and weekly iron supplementation (Cook et al., 1995; Ekström et al., 1996; Liu et al., 1996; Mumtaz et al., 2000; Ridwan et al., 1996). However, since the studies were focused in pregnancy, no information is available about the effect of different doses of iron supplementation during pregnancy and puerperium on anaemia and iron stores in postpartum period.

In 1998, we began a controlled iron supplementation trail to evaluate the efficacy and effectiveness of daily and weekly dosages of iron supplementation during pregnancy. As recommended by the government of Bangladesh the women in the trial were advised to continue iron supplementation until 6 weeks postpartum. The aim of this study is to compare the daily and weekly doses of iron supplementation during pregnancy and puerperium on haemoglobin concentration and iron status at 6 weeks postpartum.

Subjects and methods

Study area and setting

The study was conducted through 1997 and 1998 in Mymensingh district, Bangladesh, the Central part of the country. In the study area, the population density, illiteracy and malnutrition are high, has a plain agricultural land and limited access to health service, all comparable to rest of the country. To support the government policy related to iron supplementation in Bangladesh, BRAC distributes iron tablets to the pregnant women in the study area through its community-based antenatal care centres (ANCCs). Each ANCC covers about 1000 population and managed by a female Community Health Worker (CHW).

Randomisation

Fifty ANCCs of BRAC were randomly assigned either to the daily or to the weekly doses of iron supplementation. Each woman in the daily supplemented group was advised to take one iron tablet daily containing 60 mg of elemental iron (as ferrous sulfate) and 250 μg of folic acid during or after dinner. In the weekly supplemented group, each woman was advised to take two iron tablets once a week (every Friday), one during brake fast and the other during dinner. Thus, every woman in the daily group received 420 mg of elemental iron per week as opposed to 120 mg in the weekly group. All the supplements had same red colour and shape and were produced in Norway. The women received the supplements from second trimester of pregnancy (average fundal height 18 cm) and were asked to continue until 6 weeks postpartum.

Subjects

All pregnant women in the study area who had fundal height 12-22 cm, yet to start taking iron supplements during the current pregnancy and had haemoglobin concentration ≥80 g/L were considered eligible to participate in the iron supplementation trial. Informed consent was obtained from each woman at enrolment.

Through household visits, the eligible women were identified and information were collected on their socio-economic situation (SES) and reproductive history. Their registration at the monthly antenatal care activities was encouraged. At the day of enrolment, first four or five women meeting the inclusion criteria were recruited from each centre. Thus, 209 pregnant women were recruited in the iron supplementation trial. The number of women recruited at each centre varied between 4 to 6, but at most centres it was 4.

Methods

Characteristics such as age, gestational age, parity, family size, schooling, household land ownership, and perceived household economic status were collected on all identified women through house-to-house visits. Fundal height in cm was measured to estimate gestational age. The questionnaire included three indicators of SES: formal schooling of the woman, household landholding and perceived household economic status. A SES score was constructed using a combination of the three SES indicators. The score ranged from 0 to 3 based on the accumulated number of negative attributes. For example, a woman who

reported that she had no formal schooling, was economically deficit, and had <0.5 acre of land, was given a SES score of 0.

The intake of iron tablet was monitored by use of a special pill bottle called the Medication Event Monitoring System or MEMS® (Cramer et al., 1986). A microprocessor was embedded in the bottle cap to record time and date each time it was opened. The MEMS counted all the events of opening and closing occurring within 10 minutes as a 'single event'. No such event was recorded if the cap was not properly closed. Each recorded event was considered as a proxy of one tablet intake. The women were blinded about the real function of the MEMS. At the end of the three-month supplementation, all MEMS were collected and replaced by an ordinary cap. The women were arbitrarily grouped by the number of iron tablets taken in 11 weeks, i.e., <40 tablets and ≥40 tablets, which was about half of the recommended dose in the daily group. This arbitrary grouping of tablet intake was considered as a proxy indicator of tablet intake for the complete supplementation period.

Haemoglobin concentration was determined twice, at recruitment and at 6th week postpartum, from a drop of venous blood by use of the HemoCue® portable haemoglobinometre. The HemoCue method has been proven to be comparable in accuracy and precision with the standard cyanmethemoglobin method in other countries including Bangladesh (Johns & Lewis, 1989; Morris et al., 1999). The accuracy of the HemoCue machines was checked daily using the control cuvettes provided with the machines. A haemoglobin concentration <110 g/L was defined

as anaemia during pregnancy and <120 g/L during postpartum (non pregnant women) (WHO, UNICEF & UNU, 1998).

At recruitment and at 6 weeks postpartum, venous blood was collected in an untreated evacuated tube and was transported on ice to the field laboratory within 4 h. The blood was centrifuged at the field laboratory at room temperature for 5 minutes at 2264-2430 x g and the serum was taken off and frozen at -20^{0}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^{0}C. At the end of the study the samples were transported to the USA on dry ice and laboratory analysis was performed at the Department of Nutrition, University of California, Davis. Serum ferritin (SFt) was assessed using radioimmunoassay (RIA) (Diagnostic Products, San Diego, CA). SFt values <12 µg/L were considered to reflect depleted iron stores (Dallman et al., 1996). Soluble serum transferrin receptors (sTfR) were assessed by an enzyme-linked immunosorbent assay (ELISA) (Ramco Laboratories, Houston, TX). sTfR values >8.5 mg/L were considered indicative of tissue iron deficiency (Carriaga et al., 1991).

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

Haemoglobin concentration was normally distributed and mean was used as a measure of central tendency. For analysis, SFt and sTfR were normalised by a natural logarithmic transformation and medians were used as a measure of central tendency. To detect difference between two independent groups, Student's *t* test and Kruskal-Walls test were used. Paired t-test and Wilcoxon Signed Rank teat were used to detect changes in haemoglobin concentration and iron status from baseline to 6 weeks postpartum. Chi square test was used to study the association between categorical variables. During analysis, statistical significance was defined as $P<0.05$. Data were analysed by using SPSS for WINDOWS, Release 7.5.1 (SPSS Inc, Chicago).

Results

Two hundred and sixteen pregnant women were initially identified and considered for recruitment in the iron supplementation trial. Two hundred and nine were finally recruited. Fifty-one women were lost to follow-up at 6 weeks postpartum. Thus, the study women consisted of 158 subjects who had data on haemoglobin concentration and iron status both at baseline and at six weeks postpartum. Of them, 133 women had data on iron tablet intake for 11 weeks during pregnancy **(Table 1)**

The study women were older and had higher fundal height compared to those who were lost to follow-up **(Table 2)**. There was no difference between these two groups in terms of parity, SES, haemoglobin concentration and iron status. Among the study women comparison of the baseline characteristics was made

between the daily and the weekly supplemented groups. There was no difference between these two groups. Similarly, of the study women there was no difference between women who took <40 iron tablets in 11 weeks compared to those who took ≥40 iron tablets. Among the women who were lost to follow up, SES was somewhat better in the weekly compared to the daily group (P=0.07), but other baseline characteristics were found to be similar.

After continuous iron supplementation through pregnancy and puerperium, the daily and the weekly supplementation groups did not show any significant difference in haemoglobin concentration and iron status at 6 weeks postpartum. Similarly, the daily and the weekly supplementation groups did not differ for the prevalence of anaemia, depleted iron stores (low SFt) and tissue iron deficiency (high sTfR) (**Table 3**). In the 11 weeks of the monitored supplementation, median (mean ± SD) iron tablet intake was 58 (50 ± 27) in the daily group and 23 (23 ± 10) in the weekly group. (**Fig 1 & 2** *histograms of iron tablet consumed by daily and weekly groups be added in the result*).

Overall, the mean±SD haemoglobin concentration increased from 111 ± 15 g/L at baseline to 130 ± 17 g/L at 6 weeks postpartum (P<0.001). Similarly, median SFt increased (14.7 µg/L to 57.1 µg/L, P<0.001), and sTfR decreased (6.3 mg/L to 4.9 mg/L, P=0.12) during the same period. At 6 weeks postpartum, 26.7% of the anaemic women at baseline remained anaemic and 21.7% of the non-anaemic women at baseline developed anaemia.

The haemoglobin concentration and iron status data were compared between the two groups of women who took fewer and 40 or more iron tablets in 11 weeks during pregnancy (**Table 4**). Intake of the tablets ≥40 was found to be significantly associated with increased haemoglobin concentration and SFt ($P<0.05$). None of the baseline characteristics differed between the two supplementation groups.

Discussion

Main finding

The major objective of the study was to compare the effectiveness of the daily and weekly doses of iron supplementation during pregnancy and puerperium on haemoglobin concentration and iron status at six weeks postpartum. The effectiveness did not differ between the daily and the weekly supplementation groups. An overall increase in haemoglobin concentration and iron status was observed, which is in agreement with studies conducted elsewhere (Zimmer et al., 1998; Preziosi et al., 1997; Taylor & Lind, 1981). Women associated with a high iron tablet intake had significantly better status compared to those who took fewer tablets.

Validity

Difference in the baseline characteristics among women in the daily and weekly supplemented groups could have biased the final haemoglobin concentration and iron status results. Comparison of the baseline characteristics in the study women revealed no significant difference between the two supplementation groups. Furthermore, the baseline characteristics of women who took <40 iron tablets did not differ from those taking ≥40 tablets. Thus, we conclude that the effect of the daily and the weekly doses of iron supplementation at 6 weeks postpartum were not possibly biased by difference in any of the baseline characteristics.

The study women were older and had higher fundal height than those lost to follow up at 6 weeks postpartum period (**Table 2**). Other baseline characteristics

such as parity, SES, haemoglobin concentration and iron status did not show any significant difference. As found in our study, fundal height had inverse correlation with haemoglobin concentration and SFt ($P<0.05$), but not with sTfR. It supports the earlier findings that haemoglobin concentration and SFt fall with increased gestational age (Taylor et al., 1982; Puolakka et al., 1980) largely due to expansion of plasma volume (Hytten, 1985), but sTfR is unaffected by pregnancy (Åkesson et al., 1998). However, age was not found to be correlated with any of the indicators of haemoglobin concentration and iron status. Thus, although not conclusive without a control group, inclusion of women with higher gestational age in the present study could have underestimated the overall response of the iron supplementation at 6 weeks postpartum.

Although not verified, the MEMS has been found useful to monitor the number of tablets taken in a long-term medical therapy elsewhere (Cramer et al., 1989). In our study, data on iron tablet intake in 11 weeks was used as a proxy of the intake during the complete supplementation period. The MEMS recorded the events of opening and closing. It did not provide information on the actual number of tablet (s) taken out from the bottle during each event as well as if the tablet (s) was really ingested. Therefore it might result in over- and/or under-estimation of the actual number of tablets taken. In this study, however, an attempt was made to minimise the over-estimation by excluding all events recorded on the first day in the analysis. It was assumed that the women would open the bottle more frequently on that day to fulfil their curiosity about the new device, thus resulting in recording of additional events.

Daily and weekly issue

In our study, the women assigned to daily and weekly groups showed no difference in haemoglobin concentration and iron status at 6 weeks postpartum. Similar findings of lack of difference in effect during pregnancy have been reported (Ridwan et al., 1996; Liu et al., 1996; Cook et al., 1995; Schultink et al., 1993). In the context of our present study, three alternative possibilities may be discussed to explain the reasons why no difference was found between the daily and weekly supplemented groups.

The first possibility is that women neither in the daily nor in the weekly supplemented group responded to iron supplementation. The observed increase in haemoglobin concentration and iron status was largely due to other factors, such as, reversal of haemodilution at late pregnancy (DeLeeuw et al., 1966) and positive iron balance during lactation (Hallberg, 1992). Without a control group it is difficult to evaluate the contribution of the supplementation in pregnant women. However, the comparison of tablet intake suggests that a significant effect can be attributed to iron supplementation and thus, a lack of response may not explain the lack of difference between the daily and weekly doses of iron supplementation.

The second possibility is that iron tablet intake was not as different as expected between women in the daily and the weekly supplemented groups and both the dose schedules produced similar response at 6 weeks postpartum. Although we did not collect information on iron tablet intake during the entire supplementation period, it was shown from the 11 weeks' intake data that 40%

women in the daily supplemented group took less than 40 tablets, which is about half of the recommended dose in that group. On the other hand, 50% women in the weekly supplemented group took more than 22 tablets, which is 100% of the recommended dose. This phenomenon resulted in similar number of iron tablets taken by some women in the daily as well as in the weekly supplemented groups, producing an equal response in haemoglobin concentration and iron status at 6 weeks postpartum.

The third possibility is that women in the daily supplemented group had higher number of tablet intake than the weekly supplemented group, but both produced a similar response at 6 weeks postpartum. As recommended, each woman in the daily supplemented group should have consumed 77 tablets in 11 weeks compared to 22 tablets in the weekly supplemented group. Although some women in both the groups took lower number of tablets than what was recommended, we have observed that on average women in the daily supplemented group took higher number of iron tablets (50 tablets) than in the weekly supplemented group (23 tablets).

Thus, the lack of difference in the response between the two supplementation groups as reported in our study is possibly attributed to an overlapping of the second and the third possibilities. Lower number of iron tablets taken by some women in the daily supplemented group as well as higher intake by some women in the weekly supplemented group possibly have attributed to the finding of no difference in response between the two supplementation groups. Had majority of the women in the daily-supplemented group took ≥40 tablets, they could have

demonstrated a significantly higher response in haemoglobin concentration and iron status than the weekly supplemented group at 6 weeks postpartum.

Other issue

In our study, 22% of the non-anaemic women and 27% of the anaemic women at baseline had anaemia at 6 weeks postpartum. Both the prevalence figures did not differ between the supplementation groups. Among women who were non-anaemic at baseline, iron tablet intake was found to be lower in women who developed anaemia compared to those who did not develop anaemia at 6 weeks postpartum. A similar trend of lower iron tablet intake was observed in women who were anaemic at baseline and remained anaemic at 6 weeks postpartum. Thus, lesser number of iron tablet intake during pregnancy and puerperium largely explains the major contribution of anaemia in iron supplemented women at 6 weeks postpartum.

Conclusion

In conclusion, there is no difference between the daily and the weekly doses of iron supplementation during pregnancy and puerperium on haematology and iron status at 6 weeks postpartum. Iron tablet intake of the target group should be maximised. Further research is needed to evaluate the number of iron tablets required during pregnancy and puerperium to produce and sustain a haematological response including the effect of different delivery systems in a primary health care setting for maximising the compliance.

References *(not all be included, some yet to be formatted)*

Agarwal KN, Agarwal DK & Mishra KP (1991): Impact of anaemia prophylaxis in pregnancy on maternal haemoglobin, serum ferritin and birth weight. Indian. J. Med. Res. **94**, 277-280.

Åkesson A, Bjellerup P, Berglund M, Bremme K & Vahter M (1998): Serum transferrin receptor: a specific marker of iron deficiency in pregnancy. Am. J. Clin. Nutr. **68**,1241-1246.

Antelman G, Msamanga GI, Spiegelman D, Urassa EJN, Narh R, Hunter DJ & Fawzi WW (2000): Nutritional factors and infectious disease contributes to anemia among pregnant women with human immunodeficiency virus in Tanzania. J. Nutr. **130**,1950-1957.

Bangladesh Bureau of Statistics (1998): Report of the poverty monitoring survey. Dhaka: Bangladesh Bureau of Statistics.

Beaton GH & McCabe GP (1999): Efficacy of intermittent iron supplementation in the control of iron deficiency anaemia in developing countries: an analysis of experience. Ottawa: Micronutrient Initiative.

Bondevik GT, Eskeland B, Ulvik RJ, Ulstein M, Lie RT, Schneede J & Kvale G (2000): Anemia in pregnancy: possible causes and risk factors in Nepali women. Eu. J. Clin. Nutr. **54**, 3-8.

Bothwell TH & Charlton RW (1984): Iron deficiency in women, 2nd ed. Washington D.C: International Nutritional Anemia Consultative Group.

Bothwell TH, Charlton RW, Cook JD & Finch CA (1979): Iron metabolism in man. Oxford: Blackwell Scientific Publications.

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

Carriaga MT, Skikne BS, Finley B, Culter B & Cook JD (1991): Serum transferrin receptor for the detection of iron deficiency in pregnancy. Am. J. Clin. Nutr. **54**,1077-1081.

Chanarin I & Rothman D (1971): Further observation on the relation between iron and folate status in pregnancy. Br. Med. J. **2**, 81-4.

Charoenlap P & Dhanamitta S (1988): A WHO collaborative study on iron supplementation in Burma and Thailand. Am. J. Clin. Nutr. **47**, 280-297.

Cook JD, Dassenko SA & Lynch SR (1991): Assessment of the role of non-heme-iron availability in iron balance. Am. J. Clin. Nutr. **54**, 717-722.

Cook JD & Reddy MB (1995): Efficacy of weekly compared with daily iron supplementation. Am. J. Clin. Nutr. **62**, 117-20.

Cramer J, Mattson R, Prevey M, Scheyer R & Quellette V (1989): How often is medication taken as prescribed? JAMA **261**, 3273-3277.

Dallman PR (1987): Iron deficiency and the immune response. Am. J. Clin. Nutr. **46**, 329-334.

Dallman PR, LookerAC, Johnson CL & Carrol M (1996): Influence of age on laboratory criteria for the diagnosis of iron deficiency anemia and iron deficiency in infants and children. In *Iron Nutrition in Health and Disease,* ed. Hallberg L & Asp, N.G, pp 65-74. London: John Libbey & Company.

Dallman PR (1982): Manifestation of iron deficiency. Semin. Hematol. **19**, 19-30.

Deleeuw NKW, Lowenstein L & Hsieh YS (1966): Iron deficiency and hydremia in normal pregnancy. Medicine (Baltimore) **45**:291.

DeMaeyer EM (1989): Preventing and controlling iron deficiency anaemia through primary health care. A guide for health administrators and programme planners. Geneva: World Health Organization.

Dreyfuss M (1998): Anemia and iron deficiency during pregnancy: etiologies and effects on birth outcome in Nepal. PhD dissertation. Baltimore: Johns Hopkins University.

Dreyfuss ML, Stoltzfus RJ, Pradhan EK, LeClerq SC, Khatry SK, Shrestha SR, Katz J, Albonico M & West KP Jr (2000): Hookworks, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J. Nutr. **130**, 2527-2536.

Duthie SJ, King PA, To WK, Lopes A & Ma HK (1991): A case controlled study of pregnancy complicated by severe maternal anaemia. Aust. NZ. J. Obstet. Gynaecol. **31**, 125-127.

Ekström E-C, Kavishe Fp, Habicht JP, Frongillo E, et al. (1996): Adherence to iron supplementation during pregnancy in Tanzania: determinants and hematologic consequences. Am. J. Clin. Nutr. **64**, 368-374.

Hallberg L (1988): Iron balance in pregnancy. In *Vitamins and minerals in pregnancy and lactation*, ed. Berger H, Vol 16, pp 115-127. New York: Raven Press.

Hallberg L, Brune M & Rossander L (1989): Iron absorption in amn: ascorbic acid and dose-dependent inhibition by phytate. Am. J. Clin. Nutr. **49**, 140-144.

Helen Keller International & Institute of Public Health Nutrition (1999): Iron deficiency anaemia throughout the lifecycle in rural Bangladesh. Dhaka: Helen Keller International & Institute of Public Health Nutrition.

Hyder SMZ, Persson LÅ, Chowdhury AMR & Ekström E-C (2000): Anaemia among non-pregnant women in rural Bangladesh. Public Health Nutr. (in press).

Hytten F (1985): Blood volume changes in normal pregnancy. Clin. Haematol. **14**, 601-612.

Jahan K & Hossain M (1998): Bangladesh national nutrition survey, 1995-96. Dhaka: Institute of Nutrition and Food Science, University of Dhaka.

Johns WL & Lewis SM (1989): Primary health screening by haemoglobinometry in a tropical community. Bull. WHO. **67**, 627-33.

Klebanoff MA, Shiono PH, Selby JV, Trachtenberg AI & Graubard BI (1991): Anemia and spontaneous preterm birth. Am. J. Obstet. Gynecol. **164**, 59-63.

Kuizon M, Desnacido J, Platon T, Ancheta L & Macapinlac M (1983): Iron supplementation using different dose levels in pregnant Filipinos. Nutr. Res. **3**, 257-264.

Kurhade GA, Khanorkar SV, Puranik BM, Kher JR, Patwardhan SA & Agarwal S (1994): Serum level of iron and transferrin in pregnancy and postpartum period. Indian J. Physiol. Pharmacol. **38**, 34-38.

Lao TT, Lee CP & Mak WP (1996): Postpartum anaemia is not related to maternal iron status in the third trimester. Eur. J. Obstet. Gynecol. Reprod. Biol. **38**, 119-124.

Levin HM (1986): A benefit-cost analysis of nutritional programs of anaemia reduction. Res. Observ. **1**, 219-245.

Liu XN & Liu PY (1996): The effect of weekly iron supplementation regimen in improving the iron status of Chinese children and pregnant women. Biomedical and Environmental Sciences **9**, 341-347.

Menendez C, Todd J & Alonso P, et al. (1994): The effects of iron supplementation during pregnancy given by traditional birth attendants on the prevalence of anaemia and malaria. Trans. R. Soc. Trop. Med. Hyg. **88**, 590-593.

Micronutrient Initiative & International Nutrition Foundation (1998): Preventing iron deficiency in women and children: background and consensus on key technical issues and resources for advocacy, planning and implementing national programmes. Ottawa: Micronutrient Initiative.

Milman N, Agger AO & Nielsen OJ (1994): Iron status markers and serum erythropoietin in 120 mothers and newborn infants. Acta. Obstet. Gynecol. Scand. **73**, 200-204.

Morris SS, Ruel MT, Cohen RJ, Dewey KG, Briére de la B & Hassan MN (1999): Precision, accuracy and reliability of hemoglobin assessment with use of capillary blood. Am. J. Clin. Nutr. **69**, 1243-1248.

Mumtaz Z, Shahab S, Butt N, Rab MA & DeMuynck A (2000): Daily iron supplementation is more effective than twice weekly iron supplementation in pregnant women in Pakistan in a randomized double-blind clinical trial. J. Nutr. **130**, 2697-2702.

Murphy JF, O'Riordan J, Newcombe RJ, Coles EC & Pearson JF (1986): Relation of haemoglobin levels in first and second trimester to outcome of pregnancy. Lancet **1**, 992-995.

Preziosi P, Prual A, Galan P, Daouda H, Boureima H & Hercberg S (1997): Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am. J. Clin. Nutr. **66**, 1178-1182.

Puolakka J, Jänne O, Pakarinen A, Järvinen PA & Vihko R (1980): Serum ferritin as a measure of iron stores during and after normal pregnancy with and without iron supplements. Acta. Obstet. Gynecol. Scand. **95**, Suppl., S43-51.

Ridwan E, Schultink W, Dillon D & Gross R (1996): Effects of weekly iron supplementation on pregnant Indonesian women are similar to those of daily supplementation. Am. J. Clin. Nutr. **63**, 884-90.

Sen B & Begum S (1998): Methodology for identifying the poorest at local level. "Macroeconomics, Health and Development" series number 27. Geneva: World Health Organization.

Singh N, Shukla MM & Sharma VP (1999): Epidemiology of malaria in pregnancy in central India. Bull. World Health Org. 77, 567-572.

Stoltzfus RJ & Dreyfuss ML (1998): Guidelines for the use of iron supplements to prevent and treat iron deficiency anaemia. Washington D.C: International Nutritional Anaemia Consultative Group (INACG).

Suharno D, West CE, Muhilal, Karyadi D & Hautvast J (1993): Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet **342**, 1325-1328.

Thomsen JK, Prien-Larsen JC, Devantier A & Fogh-Andersen N (1993): Low dose iron supplementation does not cover the need for iron during pregnancy. Acta. Obstet. Gynecol. Scand. **72**, 93-98.

Tylor DJ & Lind T (1981): Puerperal haematological indices. Br. J. Obstet. Gynaecol. **88**, 601-606.

Viteri FE (1994): The consequences of iron deficiency and anemia in pregnancy. In *Nutrient regulation during pregnancy, lactation and infant growth*, ed. Allen L, King J & Lonnerdal B, pp 127-139. New York: Plenum.

Viteri FE (1998): Iron supplementation for the control of iron deficiency in population at risk. Nutr. Rev. **55**, 195-209.

World Health Organization (1992): The prevalence of anaemia in women: A tabulation of available information. Geneva: World Health Organization.

World Health Organization (1994): Bench aids in the diagnosis of intestinal parasites. Geneva: World Health Organization.

World Health Organization (1998) Global database on anaemia. Geneva: World Health Organization.

World Health Organization, UNICEF & United Nations University (1998): Iron Deficiency: indicators for assessment and strategies for prevention. Geneva: World Health Organization.

Yip R (1994): Iron deficiency: contemporary scientific issues and international programmatic approaches. J. Nutr. **124**, Suppl..S1479-1490.

Yip R (1996): Iron supplementation during pregnancy: is it effective? Am. J. Clin. Nutr. **63**, 853-855 (editorial).

Zimmer P, Garza C, Heller ME, Butte N & Goldman AS (1998): Postpartum maternal blood helper T ($CD3^+CD4^+$) and cytotoxic T ($CD3^+CD8^+$) cells: correlations with iron status, parity, supplement use, and lactation status. Am. J. Clin. Nutr. **67**, 897-904.

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

216	Identified subjects ⇒ 3 refused ⇒ 4 haemoglobin <80 g/L		
209	Enrolled in the iron supplementation study		
51	Lost to follow up at 6 weeks postpartum	Iron tablet dose	
		Daily	Weekly
	39 refused blood sampling	26*	13
	2 were excluded due to Hb <75 g/L	2	-
	1 still birth	-	1
	9 incomplete data on haematology	4	5
158	Subjects with complete information on haematology and iron status	72	86
133	Subjects with complete information on haematology, iron status and compliance	62	71

*Difference between daily and weekly is significant, $P<0.05$

Table 2
Baseline characteristics of the study women and women lost to follow up

	Study women (n=158)	Lost to follow-up		
		All (n=51)	Daily (n=32)	Weekly (n=19)
Age (y)[1]	24.7±5.9*	22.4±5.7	22.9±5.8	21.5±5.5
Parity (#)[1]	1.9±1.9	1.4±1.9	1.5±1.7	1.3±2.1
Fundal height (cm)[1]	17.3±2.2*	16.2±1.7	16.3±1.5	16.2±2.0
SES score[1]	1.2±0.7	1.1±0.8	1.2±0.8	0.8±0.7
Hb[2]				
Mean+sd (g/L)	111±15	109±14	110±13	107±14
Anaemia (%)	47.5	54.9	53.1	57.9
SFt[3]				
Median (µg/L)	14.7	13.7	12.7	14.9
Low SFt (%)	39.9	42.0	45.2	36.8
sTfR[4]				
Median (mg/L)	6.3	6.1	6.7	4.9
High sTfR (%)	25.9	17.6	21.9	10.5

[1]Mean±sd
*Difference between study women and lost to follow-up is significant, $p<0.05$
[2]Hb, Haemoglobin concentration: [3]SFt, Serum ferritin: [4]sTfR, Serum transferrin receptor: [5]sTfR/SFt, Ratio of sTfR and SFt.

Table 3
Haematology and iron status at baseline and 6th week postpartum by dose frequency of iron supplementation (n=158)

	Baseline♦		6th week postpartum♦	
	Daily group (n=72)	Weekly group (n=86)	Daily group (n=72)	Weekly group (n=86)
Hb				
Mean ± SD (g/L)[1]	111 ± 15	111 ± 14	129 ± 17	130 ± 17
Anaemia (%)[2]	48.6	46.5	25.0	23.3
SFt				
Median (µg/L)[3]	12.7	19.2	57.7	54.6
Low SFt (%)[2]	47.2	33.7	6.9	9.3
sTfR				
Median (mg/L)[3]	6.3	6.3	4.9	4.9
High sTfR (%)[2]	27.8	24.4	22.2	18.6

♦No significant difference between the daily and weekly groups, $P>0.05$
[1]t-test
[2]Chi-square test
[3]Kruskal-Walls test

Table 4
Haematology and iron status at baseline and 6th week postpartum by number of iron tablets taken during pregnancy (n=133)

	Baseline		6th week postpartum	
	<40 tablets group (n=90)	≥40 tablets group (n=43)	<40 tablets group (n=90)	≥40 tablets group (n=43)
Hb				
Mean ± SD (g/L)[1]	111 ± 14	111 ± 15	128 ± 18*	134 ± 16
Anaemia (%)[2]	44.0	47.6	27.8*	11.9
SFt				
Median (µg/L)[3]	19.6	13.7	48.3*	62.5
Low SFt (%)[2]	38.5	38.1	11.0	2.4
sTfR				
Median (mg/L)[3]	6.2	6.3	5.1	4.5
High sTfR (%)[2]	25.3	31.0	19.8	16.7

*Difference between 0-40 tablets and ≥41 tablets is significant, $P<0.05$
[1]t-test
[2]Chi-square test
[3]Kruskal-Walls test