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Anaemia and iron deficiency during pregnancy in rural Bangladesh

SM Ziauddin Hyder^{1,2,*}, Lars-Åke Persson³, Mushtaque Chowdhury¹, Bo Lönnerdal⁴ and Eva-Charlotte Ekström³

¹Research and Evaluation Division, BRAC, Dhaka, Bangladesh: ²Division of Gastroenterology and Nutrition, Hospital for Sick Children and Center for International Health, University of Toronto, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8: ³International Maternal and Child Health, Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden: ⁴Department of Nutrition, University of California, Davis, CA, USA

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Abstract

Objective: To study the prevalence of anaemia and its association with measures of iron deficiency (ID) among a group of pregnant women.

Design: Cross-sectional survey.

Setting: Pregnant women identified through house-to-house visits and participating in community-based antenatal care activities in a rural location of Mymensingh, Bangladesh.

Subjects: The estimates are based on 214 reportedly healthy pregnant women in their second trimester. Information on socio-economic status and reproductive history were obtained through home visits and venous blood samples were collected at antenatal care centres. Haemoglobin concentration (Hb) was measured by HemoCue, serum ferritin (sFt) by radioimmunoassay and serum transferrin receptor (sTfR) by enzyme-linked immunosorbent assay methods. ID was defined as presence of either low sFt ($< 12 \mu\text{g l}^{-1}$) or high sTfR ($> 8.5 \text{ mg l}^{-1}$).

Results: The prevalence of anaemia ($\text{Hb} < 110 \text{ g l}^{-1}$) was 50%, but severe anaemia ($\text{Hb} < 70 \text{ g l}^{-1}$) was absent. Low sFt was observed in 42%, high sTfR in 25%, either low sFt or high TfR in 54% and both low sFt and high TfR in 13% of the pregnant women. Two out of three anaemic women had an indication of ID, which was present in 80% of women with moderate ($\text{Hb } 70\text{--}99 \text{ g l}^{-1}$) and 50% with mild ($\text{Hb } 100\text{--}109 \text{ g l}^{-1}$) anaemia. Four out of 10 non-anaemic women ($\text{Hb} \geq 110 \text{ g l}^{-1}$) also had ID, but the prevalence was significantly lower than that observed in anaemic women ($P = 0.001$).

Conclusions: Despite the high prevalence of anaemia, severe cases were absent. The prevalence of ID increased at lower Hb. However, an increased prevalence was also found among women in the highest category of Hb.

Keywords
Anaemia
Iron deficiency
Serum ferritin
Transferrin receptors
Pregnancy
Bangladesh

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 affected¹. It is associated with a number of negative outcomes, such as preterm delivery, low birth weight, perinatal mortality and – for severe anaemia – maternal death^{2–5}. Iron deficiency (ID) has often been claimed to be the predominant cause of anaemia^{6,7}. Consequently, anaemia prevention and control strategies have focused on correcting this deficiency by routine iron supplementation. Other contributing factors, e.g. parasitic infestations, chronic infections and other micronutrient deficiencies, have often been disregarded.

The effectiveness of iron supplementation programmes has generally been low and recently it has been

questioned whether ID is contributing as much as earlier perceived to the prevalence and severity of anaemia⁶. It is generally not clearly known how common ID is and to what extent maternal anaemia is associated with ID.

In Bangladesh, two different surveys have estimated the anaemia prevalence among pregnant women to be 50 and 59%^{8,9}. In these surveys, blood samples were analysed only to measure haemoglobin concentrations (Hb). Therefore, information on ID and its association with maternal anaemia is not available in the country, particularly among women who are otherwise healthy. The objective of the present work was to gather information on the prevalence of anaemia and its association with measures of ID among reportedly healthy pregnant women in a rural location of Bangladesh.

*Corresponding author: Email ziauddin.hyder@sickkids.ca

Subjects and methods

Study area

The study was conducted in May–November 1997 in rural Mymensingh, north-western 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

BRAC (formerly known as Bangladesh Rural Advancement Committee), a large national private development organisation in Bangladesh, provides antenatal care services to rural pregnant women through community-based antenatal care centres (ANCCs). Each ANCC covers a population of around 1000. This study used baseline information from an iron/folate supplementation trial where 50 out of 54 ANCCs in the area were selected. The selection of these ANCCs was done at random and therefore each of the 54 ANCCs had an equal chance to be included in this study. In the catchment area of the 50 ANCCs, all pregnant women with fundal height <22 cm ($n = 611$) were identified through house-to-house visits and invited to participate. At the next monthly ANCC meeting, the first four women enrolling in the maternity programme who were initially screened and fulfilling the inclusion criteria for the supplementation trial (i.e. fundal height between 14 and 22 cm, no previous iron supplementation during the current pregnancy and reportedly healthy) were included in this study. A few centres included up to six women. The final sample consisted of 214 pregnant women who consented to participate. There was no missing information on Hb, serum ferritin (sFt) or serum transferrin receptor (sTfR).

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.

Data collection

Background information on age, parity, gestational age and socio-economic situation was collected from all identified pregnant women at the first household visit prior to their participation in the antenatal care activities. Gestational age was determined by measuring fundal height. The female research field assistants received a 3-day training programme to measure symphysis–fundal height, which was 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 foetal

crown–rump length would be reflected. The questionnaire included three indicators of socio-economic situation: formal schooling of the woman, household landholding and perceived household economic status¹⁰. Households with less than 0.5 acre of land were categorised as functionally landless¹¹. 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. This indicator to measure household economic status has been tested in earlier studies conducted in rural Bangladesh¹⁰.

Biochemical analyses

At registration for the maternity programme, a venous blood sample was collected in an untreated evacuated tube. Hb was determined in the field by use of the HemoCue[®] system. The system has been shown to have accuracy and precision similar to the standard cyanmethaemoglobin method¹². The accuracy of the HemoCue was checked daily with control cuvettes provided by the manufacturer with each machine. Hb <110 g l⁻¹ was defined as anaemia, 100–109 g l⁻¹ as mild anaemia, 70–99 g l⁻¹ as moderate anaemia and <70 g l⁻¹ as severe anaemia⁶. Within 4 h of collection, remaining blood samples were transported on ice to a field laboratory in Mymensingh. 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 the Department of Nutrition, University of California at Davis, USA. sFt was assessed using radioimmunoassay (Diagnostic Products, San Diego, CA, USA). Values <12 µg l⁻¹ were regarded to reflect depleted iron stores¹³. Soluble sTfR was assessed by an enzyme-linked immunosorbent assay (Ramco Laboratories, Houston, TX, USA). Values >8.5 mg l⁻¹ were used as indicative of functional ID¹⁴.

Stool samples were collected at the ANCCs for examination of the presence of *Ascaris* and hookworm infestations by the microscope method. The sample was diluted in sodium chloride solution and the presence of worms was evaluated by a semi-quantitative technique¹⁵. Stool samples were not available for four individuals with Hb <80 g l⁻¹, as they were excluded from the trial.

Statistical methods

Hb had a normal distribution. sFt and sTfR were not normally distributed; thus, logarithmic transformation was performed. Original values of Hb and transformed values of sFt and sTfR were used to perform all statistical tests. Trend and departure from linearity were tested by the chi-square test. Differences in the baseline characteristics between the two groups of women who had information on Hb and iron status (participated in this study) and those

who did not have this information (did not participate in this study) were tested by the independent *t*-test. Associations of Hb with the distributions of sFt and sTfR and the prevalence of low sFt and high sTfR were tested using linear regression analysis. Associations of sFt and sTfR between categories of Hb were tested by analysis of variance. Analyses were done using the SPSS for Windows software package (version 7.5.1; SPSS Inc., Chicago, IL, USA).

Results

Information was collected on socio-economic condition and reproductive history of all of the identified pregnant women with fundal height <22 cm in the study area ($n = 611$). It allowed a comparison of characteristics between pregnant women who were included in this study ($n = 214$) and those who were not ($n = 397$). These two groups of women did not differ for any of the characteristics including age, fundal height, socio-economic status and infestations with intestinal parasites. The only difference between the groups was in mean parity, which was higher in the women included in this study (2.2 vs. 1.8, $P = 0.05$).

The study women were on average 24 years old (range 14–44 years), having their first (31%), second (22%), third (17%) or more (30%) pregnancy. All of them were in their second trimester based on fundal height; mean fundal height was 17.1 cm, corresponding to 21 weeks of gestation¹⁶. Sixty-five per cent of the households were functionally landless, 72% of the women perceived themselves as economically deficit and 56% of the women had attended school for at least a year. About 38% of the women had ascariasis and three women (1%) had hookworm infestation.

Mean Hb was 110 g l^{-1} (95% confidence interval (CI) 107–111) and 50% (95% CI 43–57) of the women were anaemic. The anaemia was mild in 28% and moderate in 22% of the study women. None of them had severe anaemia. There was no association of anaemia prevalence with age, parity, fundal height or any of the three socio-economic status variables. Anaemia was not found to be associated with *Ascaris* infestation, but it was present in all three women with hookworm infestation.

The prevalence of ID showed a wide range depending on which of the indicators was used (Table 1). The prevalence was found to be highest if ID was defined as the presence of either low sFt or high sTfR (54%). However, the lowest prevalence was found if a combination of these two indicators was used (13%). ID was high in anaemic women and, although with lesser intensity, was common in non-anaemic women. In anaemic pregnant women, two in every three, and in non-anaemic women, two in every five, women had an indication of either low sFt or high sTfR or a combination of both. Either low sFt or high sTfR had no association with

Table 1 Prevalence of iron deficiency in anaemic and non-anaemic participating women. Values are expressed as % (95% confidence interval)

Iron deficiency	All ($n = 214$)	Anaemic ($n = 107$)	Non-anaemic ($n = 107$)
sTfR > 8.5 mg l^{-1}	25 (21–31)	30 (22–38)	19 (12–26)
sFt < $12 \text{ } \mu\text{g l}^{-1}$	42 (35–49)	55 (46–64)	29 (21–37)
sTfR > 8.5 mg l^{-1} and sFt < $12 \text{ } \mu\text{g l}^{-1}$	13 (9–17)	20 (13–27)	6 (2–10)
sTfR > 8.5 mg l^{-1} or sFt < $12 \text{ } \mu\text{g l}^{-1}$	54 (47–61)	65 (56–74)	42 (33–51)

sFt – serum ferritin; sTfR – serum transferrin receptor.

any of the background factors except parity ($P = 0.08$ and 0.001, respectively).

The median value of sFt was close to the cut-off for depleted iron stores, while sTfR was within the normal range (Table 2). Hb was significantly associated with the distributions of sFt and sTfR ($P = 0.000$ and 0.002, respectively), as well as with the prevalence of low sFt and high sTfR ($P = 0.000$ and 0.004, respectively). Medians of sFt increased and sTfR decreased from low to high Hb. Similarly, the prevalence of low sFt and high sTfR also showed a significant decrease with increasing Hb. The proportion of women with indication of low sFt, high sTfR or a combination of both seemed to decrease with increasing Hb (Fig. 1). This tendency was consistent for the presence of high sTfR (test for trend, $P < 0.01$; departure from linearity, $P = 0.97$). However, although the prevalence of low sFt decreased with increasing Hb, the proportion of pregnant women with low sFt increased significantly ($P < 0.05$) in the highest range of Hb (test for trend, $P < 0.01$; departure from linearity, $P < 0.05$). In the subgroup with Hb $120\text{--}129 \text{ g l}^{-1}$, five of 29 women had low sFt, while eight of 20 among those with Hb $\geq 130 \text{ g l}^{-1}$ had a low value ($P = 0.08$) (Table 3).

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^{8,9}. 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%¹⁷. From the plains of Nepal, a prevalence of 73% with 7% being severely anaemic has been reported¹⁸. In Sri Lanka, 65% of pregnant women were anaemic¹⁹.

ID 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

Table 2 Iron status of participating women according to different degrees of anaemia ($n = 214$)

Haemoglobin (g l^{-1})	sFt ($\mu\text{g l}^{-1}$)		sTfR (mg l^{-1})	
	Median (25th, 75th percentile)	$< 12 \mu\text{g l}^{-1}$ (%)	Median (25th, 75th percentile)	$> 8.5 \text{mg l}^{-1}$ (%)
70–99 (moderate anaemia)	3.1 (0.6, 13.3)	73	7.5 (5.1, 9.6)	38
100–109 (mild anaemia)	13.4 (4.5, 29.8)	41	6.2 (5.0, 8.6)	25
≥ 110 (normal)	20.5 (10.2, 37.0)	29	5.3 (3.6, 8.0)	19
All	13.9 (5.7, 29.5)	42	6.2 (4.4, 8.5)	25
<i>P</i> -value	< 0.001	< 0.001	< 0.001	0.04

sFt – serum ferritin; sTfR – serum transferrin receptor.

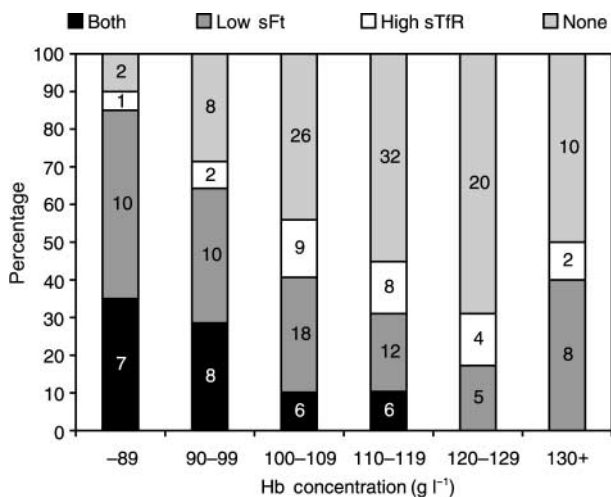


Fig. 1 Percentage of women with low serum ferritin (sFt) and high serum transferrin receptor (sTfR) at different haemoglobin (Hb) concentrations ($n = 214$, actual number of women are shown in each box)

anaemia^{20–22}. None of these causative factors was prevalent in the study area^{23,24}, and it is possible that this is the explanation for the lack of severe anaemia. Information on parasite infestation was lacking for four individuals with low Hb. If all these had hookworm infestation its prevalence would still have been low, 3%.

Forty-two per cent of the women had low sFt concentration, indicating insufficient iron stores. This

may both be an underestimation and overestimation of this type of ID. Inflammations, which are common in countries like Bangladesh, can elevate sFt concentration and lead to underestimation of the proportion with low iron stores^{25–27}. The deficiency may also be overestimated as sFt normally decreases after the first trimester of pregnancy²⁸.

Soluble sTfR has been suggested to be a more reliable indicator of ID since it is not affected by infection^{25,26,29}. The prevalence of high sTfR was 25% and thus fewer women were functionally affected by ID. 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 deficiency³⁰. It has been reported that 49% of pregnant women in Bangladesh have serum retinal concentration $< 1.05 \mu\text{mol l}^{-1}$, with the prevalence of night blindness being 2.7%³¹.

An unexpected finding is the seemingly high prevalence of low sFt among women in the highest Hb 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 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 ID and keep the possibility open that even women with high Hb may be a

Table 3 Prevalence of low serum ferritin (sFt $< 12 \mu\text{g l}^{-1}$) and high serum transferrin receptor (sTfR $> 8.5 \text{mg l}^{-1}$) by haemoglobin concentration category. Values are expressed as n/N (%)

Haemoglobin (g l^{-1})	sFt $< 12 \mu\text{g l}^{-1}$ *	sTfR $> 8.5 \text{mg l}^{-1}$ †	sTfR $> 8.5 \text{mg l}^{-1}$ and sFt $< 12 \mu\text{g l}^{-1}$	sTfR $> 8.5 \text{mg l}^{-1}$ or sFt $< 12 \mu\text{g l}^{-1}$
< 90	17/20 (85)‡§	8/20 (40)§	7/20 (35)‡§	18/20 (90)‡§
90–99	18/28 (64)	10/28 (36)	8/28 (29)	20/28 (71)
100–109	24/50 (41)	15/59 (25)	6/59 (10)	33/59 (56)
110–119	18/58 (31)	14/58 (24)	6/58 (10)	26/58 (45)
120–129	5/29 (17)	4/29 (14)	0/29 (0)	9/29 (31)
130 +	8/20 (40)¶	2/20 (10)	0/20 (0)	10/20 (50)

* Regardless of sTfR status.

† Regardless of sFt status.

‡ Pearson chi-square, $P < 0.05$.

§ Linear-by-linear trend, $P < 0.05$.

¶ Departure from linearity, $P < 0.05$.

risk group for ID. The question is, would these women benefit from iron supplementation or not?

To our knowledge, there is no previously published information on sFt or sTfR among pregnant women in Bangladesh with which to compare our results. 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 $16 \mu\text{g l}^{-1}$ and prevalence of low sFt was 57%^{19,32}. In India, pregnant anaemic women had a mean sFt concentration of $20 \mu\text{g l}^{-1}$ (low, 51%)³³. The corresponding figures for the non-anaemic women were $27 \mu\text{g l}^{-1}$ and 15% with low sFt. Among the anaemic women, as many as 86% were reported to have high sTfR concentration. On the contrary, the prevalence of ID was considerably lower in a presumably well-nourished group of Swedish pregnant women. In this population, median sFt was $34 \mu\text{g l}^{-1}$ (low sFt, 10%) and median sTfR was 4.1 mg l^{-1} (high, 11%)³⁴. Pregnant women in Bangladesh appeared to have a similar high prevalence of ID as elsewhere in the region, i.e. of a substantially larger magnitude than that among women in a high-income country, such as Sweden.

While the high prevalence of ID suggests that iron supplementation may be an efficacious means to improve Hb, it is not yet clear to what extent indicators of ID such as low sFt and high sTfR also predict response to and, more importantly, health benefits from the supplementation. Despite ID that warrants iron supplementation, there may be other factors that limit Hb response to iron supplementation, such as deficiencies of vitamin A, vitamin B₁₂ or folic acid, and chronic infection^{35,36}.

The Bangladesh Integrated Nutrition Project and the succeeding National Nutrition Programme aim to provide all pregnant women in Bangladesh with iron/folic acid supplement³⁷. In line with the previous international iron/folate recommendation³⁸, 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 continuing until 6 weeks postpartum. In general, such large-scale efforts to prevent and control anaemia have met with limited effectiveness³⁹. This has contributed to the discussion of alternative strategies to increase effectiveness. One of the most recent contributions is the proposition that severe anaemia and ID should be treated as separate entities and dealt with separately⁴⁰. 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 programmes.

For programmes aimed at treating and preventing anaemia during pregnancy in Bangladesh, there appear 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 ID. Furthermore, in such analyses, response to iron supplementation should not be restricted to assessments of increased Hb and improved iron status as these measures may not fully reflect response in terms of improved maternal health and pregnancy outcome.

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