1 Anaemia and iron deficiency in rural Bangladeshi pregnant women living in areas of high and low
2 iron in groundwater
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Faruk Ahmed^{1,2*}, Moududur Rahman Khan³, Najma Shaheen³, Kazi Matin Uddin Ahmed
6 ⁴, Aziz Hasan⁴, Ireen Akhtar Chowdhury⁵ and Rafiqul Chowdhury⁶
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8

9 ¹Public Health, School of Medicine, Griffith University, Gold Coast, Australia

10 ²Menzies Health Institute Queensland, Griffith University, Queensland, Australia

11 ³Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh.

12 ⁴Department of Geology, University of Dhaka, Dhaka, Bangladesh.

13 ⁵Unicef, Dhaka, Bangladesh. ⁶Institute of Statistical Research and Training, University of
14 Dhaka, Dhaka, Bangladesh
15
16
17

18 ***For correspondence:**
19

20 Associate Professor Faruk Ahmed

21 Public Health, School of Medicine and Menzies Health Institute Queensland, Griffith

22 University, Gold Coast Campus, QLD 4220, Australia

23 Telephone: 61 (0) 7 5552 7874
24

25 **E-mail:** f.ahmed@griffith.edu.au
26

27 **Running Title:** Anaemia in rural Bangladeshi pregnant women
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1 **Highlights:**

- 2 • There is a high prevalence of anaemia among pregnant women living in areas of
- 3 low iron in groundwater.
- 4 • Prevalence of iron deficiency is low among pregnant women living in high
- 5 groundwater iron areas.
- 6 • Pregnant women with iron deficiency has significantly higher odds of anaemia.
- 7 • Iron deficiency is associated with daily iron intake from drinking water.

10 **Abstract**

11 **Background:** Recent studies found a low rate of iron deficiency in Bangladeshi non-pregnant
 12 and non-lactating women. This was attributed to high iron concentration in drinking water.
 13 However, there is limited data on iron deficiency among pregnant women in Bangladesh.

14 **Objectives:** To investigate the prevalence of anaemia, iron deficiency and iron deficiency
 15 anaemia (IDA) among rural pregnant women and explore the association of groundwater iron
 16 concentration with anaemia and iron deficiency in this group.

17 **Methods:** This study used data from a baseline assessment of an intervention study on rural
 18 pregnant women (n = 522), gestational age ≤ 20 weeks, living in areas of low and high iron in
 19 groundwater.

20 **Results:** Overall, 34.7% of the pregnant women had anaemia, 27% had iron deficiency, and
 21 13.4% had IDA. Prevalence of anaemia, iron deficiency and IDA among the pregnant women
 22 living in low groundwater iron areas was significantly higher than among the pregnant
 23 women from high groundwater iron areas. The odds of iron deficiency were significantly
 24 lower among pregnant women in the higher quartiles of daily iron intake from drinking
 25 water.

26 **Conclusions:** This study shows a differential prevalence of anaemia and iron deficiency
 27 among pregnant women living in areas of high and low groundwater iron. Iron status was
 28 independently associated with daily iron intake from drinking water. However, a significant
 29 proportion of the anaemia could not be attributed to iron deficiency. Further research to

1 identify other nutritional and non-nutritional contributors to anaemia in Bangladesh is needed
2 to formulate effective prevention and control programmes for anaemia.

3 **Keywords:** Anaemia, Iron deficiency, Pregnant women, Groundwater iron, Bangladesh.

7 Introduction

8 Anaemia is considered a significant public health problem among children and
9 women in Bangladesh [1]. The Bangladesh Health and Demographic Survey (BDHS) of 2011
10 reported a 50% prevalence of anaemia among pregnant women [2]. Causes of anaemia are
11 multiple and complex, but the general consensus is that half of the anaemia cases in women
12 worldwide are attributable to iron deficiency [3]. The main cause of deficiency is
13 consumption of cereal-based diets, which provide non-haem iron of poor bioavailability [4].
14 In Bangladesh, iron deficiency is thought to be the primary cause of anaemia, and a routine
15 iron-folic acid (IFA) supplementation programme for prevention of anaemia and iron
16 deficiency among pregnant women has been running for the past few decades [5]. The
17 National Micronutrient Survey 2011–2012 (NMS 2011–2012) found a very low prevalence
18 of iron deficiency (7.1%) and iron deficiency anaemia (4.8%, IDA) among non-pregnant and
19 non-lactating (NPNL) women [6]. However, there is no data on iron deficiency in pregnant
20 women in the country. The NMS 2011–2012 found that dietary iron provided only 41% of
21 the recommended dietary allowance of iron for NPNL women [6]. The survey also found a
22 significantly higher mean serum ferritin level among NPNL women living in areas of high
23 concentrations of iron in groundwater than among those living in areas of low iron in
24 groundwater [6]. A small-scale study of women of reproductive age in Bangladesh found no
25 IDA among them but observed a positive association of daily iron intake from drinking water
26 with plasma ferritin and total body iron [7]. These studies attributed the low prevalence of
27 iron deficiency and IDA to drinking iron-containing groundwater from tube wells [6,7].

Almost all rural Bangladeshis use groundwater from tube wells for drinking and cooking [8]. It is apparent that there are varying concentrations of dissolved iron in groundwater of Bangladesh. Iron concentrations in groundwater in some parts of the country are noticeably high [9-11]. An article based on NMS 2011–2012 data [9] reported a differential prevalence of anaemia and iron deficiency in Bangladeshi NPNL women living in areas of high and low iron in groundwater [12]. However, a limitation of this study was that the iron concentrations in groundwater were not measured. Areas of high and low iron in groundwater were identified using data from a different study.

The evidence of very low IDA in NPNL women [6] may have serious implications for the current IFA supplementation programme for pregnant women in the country, especially in high groundwater iron areas. However, it is also important to recognize that iron need increases significantly during pregnancy [13, 14]. It is difficult to predict the iron status of pregnant women from the findings in NPNL women. Further, considering the additional iron requirement during pregnancy, it may not be proper without sufficient evidence to assume that the Bangladeshi pregnant women living in areas of high iron in groundwater can maintain satisfactory iron status throughout pregnancy. Therefore, the present study was undertaken to investigate the prevalence of anaemia, iron deficiency and IDA in pregnant women living in areas of high and low iron in groundwater in Bangladesh. The association of groundwater iron concentration with anaemia and iron deficiency in this group was also explored by measuring iron concentration in the water they use for drinking and other household purposes.

Methods

Subjects

This study was conducted with pregnant women of gestational age ≤ 20 weeks, living in rural areas of predominantly low and predominantly high iron in groundwater. Those who had already visited the antenatal clinic (ANC) for a check-up during the current pregnancy were excluded from the study. The study was carried out in April and May 2015 for baseline assessment of an intervention study intended to examine the effect of routine IFA supplementation on anaemia and iron status.

Selection of study area

Two upazilas (sub-districts, Sharishabari and Pirgachha) from predominantly high groundwater iron areas and two upazilas (Lalmohon, Badarganj) from areas of predominantly low groundwater iron areas were selected, purposively consulting groundwater iron concentration data from the Arsenic Contamination of Groundwater in Bangladesh (ACGB) survey report [9]. First, the tube wells in the selected sub-districts were identified in the ACGB survey data file, and mean iron content of water of tube-wells in each sub-district was calculated. A low iron sub-district (area) was defined by mean values lower than the national median (1.1 mg/L) found by the ACGB survey, and mean values ≥ 1.1 mg/L were considered high. A total of 24 unions (administrative units, consisting of a cluster of villages), six unions from each upazila, were randomly selected, and then a convenience sampling was applied to recruit the pregnant women. The research proposal was approved by the Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh, and the Bangladesh Medical Research Council, Dhaka, Bangladesh.

Subject selection and data collection

The field staff identified pregnant women by a door-to-door visit and listed all prospective subjects based on the date of last menstrual period (LMP) and the ANC visit during their current pregnancy. The purpose and exact nature of the study was explained to all eligible pregnant women. They were then invited to attend the ANC on a pre-set date for data and blood collection. On the day of data collection, the purpose of the study was again explained, and those who agreed to participate were requested to sign or put thumb impression on a consent form. A total of 530 pregnant women (268 from low and 262 from high groundwater iron areas) participated in the study. The response rate was over 90%. All pregnant women who consented were tested for pregnancy by a commercial pregnancy detection kit.

All recruited women were interviewed by trained interviewers at the ANC before blood collection for socioeconomic and pregnancy-related information. The interviewers again checked the date of the LMP for confirmation. Information on the usual consumption pattern of selected food items rich in micronutrients [red meat (beef, goat and liver), fish (small and big), dairy (milk and milk products) and eggs, green leafy vegetables, non-leafy vegetables and seasonal fruits] was obtained by interview, using a 7-day food frequency

questionnaire (FFQ). The FFQ was adopted from the NMS 2011–2012, modified, and pre-tested in the study population, and a 30-day recall was used to obtain information on consumption of vitamin and mineral supplements.

Information about the source of the participants' drinking water was collected by a house-to-house visit. The subjects were asked whether they collected water in the drinking pot and drank shortly after collection, or treated it, that is, kept for some time in storage pots, or filtered, before drinking. The participants' usual daily consumption of water was estimated from the volume of their drinking pot and number of pots of water they usually drank daily.

Blood specimen collection

Five millilitres of venous blood were collected from each woman, using a disposable syringe by a haematological technician. A few drops of whole blood were taken on a hydrophobic plastic strip for measurement of haemoglobin, and the remaining blood was delivered in a centrifuge tube for collection of serum. Serum specimens were taken in plastic conical reaction tubes and frozen in dry ice, transported to the laboratory in Dhaka, and stored at -20°C until analysis.

Analytical procedures

Haemoglobin concentration was measured by HemoCue Hb 301 haemoglobinometer. Measurements of serum ferritin and serum C-reactive protein (CRP) were done by enzyme-linked immunosorbent assay (ELISA), using commercial kits (BioCheck, Inc., CA). Serum α 1-acid glycoprotein (AGP) was measured by the immunoturbidimetric method by using commercial kits from Roche Diagnostics on a Cobas C311 (Roche Diagnostics, Germany) analyser.

Analysis of water for iron and calculation of iron intake from drinking water

For those who used to drink water shortly after collection from the tube-well spout, a sample of water was collected from the tube well. Water samples were collected from the storage pot of the households that treated or kept the collected water before drinking. A total of 483 water samples were collected from the source (tube-well spout) and 102 samples from the storage pots. Iron concentration in water was measured by two methods, first in the field by the phenanthroline method, using a portable photometer. Then, concentrations in a subsample (30%; 160 samples collected from the tube-well spout and 28 from the storage pots)

were measured by atomic absorption spectrometry in the Dhaka University laboratory to check the reliability of the field assay results. Correlation between the results of the two methods was $r = 0.95$. Daily iron intake from drinking water by the participants was calculated by multiplying the amount of daily water intake with the iron concentration in drinking water collected from the tube-well spout or the storage pot, depending on the practice of the participant.

Statistical analysis

Statistical analysis was carried out by SPSS (version 20; SPSS Inc., Chicago) and SAS (SAS for Windows, version 9.3; SAS Institute). Eight subjects were excluded because of either incomplete data or insufficient blood samples for assessment of all biochemical parameters. Therefore, 262 subjects from areas of high iron in groundwater and 260 from areas of low iron groundwater were finally included in the analyses. Univariate analysis of frequency distribution, mean, median, standard deviation and range were calculated for selected variables. Sociodemographic and pregnancy-related characteristics of the participants by areas of residence, i.e., low or high groundwater iron areas, were compared by the Pearson chi-square test, Fisher exact test, independent t -test and Mann-Whitney U test as appropriate.

In the presence of inflammation or infection, serum ferritin can be in the normal range or elevated despite deficient store [15]. Hence, serum ferritin concentrations were adjusted for elevated CRP (≥ 10 mg/l) and AGP (≥ 1.0 g/l) concentrations by mathematical correction [16]. Distributions of haemoglobin and serum ferritin concentrations were checked by Kolmogorov-Smirnov goodness-of-fit test. Because serum ferritin concentrations were log transformed, values back-transformed into raw units are presented.

Anaemia was defined as haemoglobin concentration < 11.0 g/dL [3]. Iron deficiency was defined as serum ferritin concentration < 15.0 μ g/L [3]. IDA was defined as haemoglobin concentration < 11.0 g/dL and serum ferritin concentration < 15.0 μ g/L [3].

Prevalence of deficiencies was compared between subjects living in areas of high and low iron in groundwater by chi-square test. Mean haemoglobin and serum ferritin concentrations in the subjects residing in the two groundwater iron areas were compared by the general linear model (GLM) with adjustment for potential confounders using PROC

GENMOD with identity link function (SAS Institute). The frequency of consumption of various foods in the two groups were compared by the GLM with adjustment for potential confounders (subject's education, husband's education and occupation levels, ownership of homestead, cultivable land and household size). Data on iron intake were divided into quartiles, and similar analyses were carried out to compare the prevalence of anaemia, iron deficiency and IDA by iron intake from drinking water.

Finally, logistic regression analysis was done to determine the independent association of daily iron intake from drinking water, irrespective of groundwater iron area, with anaemia and iron deficiency, separately using PROC GENMOD with the logit link function (SAS Institute). The upazila was used as a cluster for within-cluster correlation. The odds ratios were adjusted for potential confounders, including various sociodemographic (age, subject's education, husband's education and occupation, household size and cultivable land ownership), pregnancy-related factors (gestational age and parity) and intake of red meat. Results of the regression analysis are presented as odds ratios adjusted for confounding variables.

Results

Mean (SD) age of the participants was 23.6 (4.8) years with a range from 13 to 38 years. Mean (SD) gestational age was 15.2 (2.7) weeks with a range from 7 to 20 weeks. Distributions of the participants by the gestational age and by parity in low and high groundwater iron areas were not significantly different (Table 1). A significantly higher proportion of the participants and their husbands in low groundwater iron areas were functionally illiterate than those in high groundwater iron areas. Similarly, a significantly higher proportion of the participants' husbands in low groundwater iron areas was unemployed or daily labourers and had no cultivable land (Table 1).

Table 2 shows the difference in haemoglobin and serum ferritin concentrations in pregnant women by groundwater iron area. Pregnant women living in high groundwater iron areas had significantly higher haemoglobin and serum ferritin concentrations. Overall, 34.7% had anaemia, 27% had iron deficiency, and 13.4% had IDA. Prevalence of anaemia, iron deficiency and IDA was significantly higher in the pregnant women in low groundwater iron areas (Table 2). Of the anaemic subjects, 38.7% [95% confidence interval (CI): 31.6%–

45.6%] had IDA. Prevalence of IDA was higher in pregnant women in low groundwater iron areas (51%) than in those in high groundwater iron areas (25.3%). Prevalence of high CRP and AGP levels in high groundwater iron areas was 8.8% and 1.9%, respectively, and these rates were 5.8% and 1.5%, respectively, in low groundwater iron areas.

After adjustment for various socioeconomic factors, significantly higher frequency of consumption of chicken, dairy and eggs, and fruits were observed in pregnant women living in areas of low iron in groundwater than in the pregnant women living in areas of high iron in groundwater. No significant differences were found in the frequency of consumption of red meat, fish, leafy and non-leafy vegetables between the groups.

In high groundwater iron areas, mean (SD) iron concentration in tube-well water was 4.23 mg/L (5.87 mg/L) with a median of 1.29 mg/L and in low groundwater iron areas, mean (SD) iron concentration in tube-well water was 0.93 mg/L (1.39 mg/L) with a median of 0.44 mg/L (Table 3). Almost all participants (99.4%) used tube-well water as a source of drinking water. Mean (SD) daily water consumption by the participants was 2.52 L (0.92 L), with a median of 2.4 L (Table 3). Eighty-one percent of the participants had a habit of drinking shortly after collection from the tube well. The others (18.7%) collected water in holding pots and drank after dissolved minerals had precipitated. Mean (SD) daily iron intake from drinking water of the participants in low and high iron areas were 1.77 mg/day (2.96 mg/day) and 11.11 mg/day (15.69 mg/day), respectively. Median (interquartile range) daily iron intake in low and high iron areas was 0.61 mg/day (0.26–1.82 mg/day) and 3.62 mg/day (0.91–15.15 mg/day), respectively (Table 3).

Differences in the prevalence of anaemia, iron deficiency and IDA in pregnant women by quartiles of daily iron intake from drinking water are shown in Figure 1. Prevalence of anaemia decreased with the increase in daily iron intake from drinking water. However, the differences were not statistically significant. Prevalence of iron deficiency in the third and fourth quartiles of daily iron intake groups was significantly lower than in the lowest quartile of the daily iron intake group. Further, the prevalence of iron deficiency in the highest quartile of the daily iron intake group was significantly lower than in the second quartile of the daily iron intake group only. Prevalence of IDA in the third and fourth

quartiles of daily iron intake groups was significantly lower than in the lowest quartile of the daily iron intake group.

The results of multivariate logistic regression showed that younger age (20–24 y vs. ≥ 25 y), higher education (secondary and above vs. functionally illiterate) and longer gestational age (>12 wk vs. ≤ 12 wk) had significantly higher odds of iron deficiency (Table 4). On the other hand, higher frequency of intake of red meat (≥ 3 times/wk vs. <3 times/wk) and higher intake of iron from drinking water (third and fourth quartiles vs. lowest quartile) had significantly lower odds of iron deficiency. A separate logistic regression was also carried out for anaemia. Participants with longer gestational age (> 12 wks vs. ≤ 12 wks) and iron deficiency (serum ferritin <15.0 $\mu\text{g/L}$ vs. ≥ 15.0 $\mu\text{g/L}$) had significantly higher odds of anaemia. Wives of farmers (farmer vs. unemployed/daily labourer) and the higher iron intake from drinking water group (second quartile vs. lowest quartile) had lower odds of anaemia (Table 4).

Discussion

The present study was carried out to compare the prevalence of anaemia, iron deficiency and IDA among rural women in their early pregnancy (gestational age ≤ 20 weeks), living in areas of high and low iron in groundwater. The current investigation was driven by the recent NMS 2011–2012 report, which claimed that iron deficiency might not be a major public health problem in Bangladesh [6]. The present study revealed that, overall, more than one-third of the pregnant women (34.7%) were anaemic, and more than a quarter were iron deficient, and 13.4% had IDA. Earlier, the BDHS of 2011 reported a 50% prevalence of anaemia in pregnant women in Bangladesh [2]. One reason for the difference in prevalence between the two studies might be due to methodologies in haemoglobin assessment. The BDHS used capillary blood by finger prick, which often produces imprecise results, whereas the present study used venous blood, which produces more reliable results. Another reason could be that the BDHS included any pregnant woman, whereas this study only included women in their early pregnancy when iron requirements are not yet as dramatically increased.

At present, there is no national data on the prevalence of iron deficiency and IDA in pregnant women on which to compare the findings of the present study. A small-scale study

conducted in rural northwest Bangladesh reported a prevalence of 19.3% anaemia during early pregnancy and less than 1% prevalence of iron deficiency [17]. The investigators suggested that the low prevalence of anaemia and iron deficiency in their study population might be due to using iron-rich groundwater for drinking and cooking [17]. There can be several reasons for the differences in the prevalence of iron deficiency and IDA between the present study and the previous study. First, their study was carried out in an area with a very high level of groundwater iron, whereas the present study covered both high and low groundwater iron areas. Second, in the present study, the median gestational age of the subjects at the time of recruitment was 15.3 weeks with an interquartile age from 13 to 17 weeks, whereas the median gestational age of the subjects in the northwest Bangladesh study was 10 weeks, with an interquartile range of 8–12 weeks. Third, the study used a more stringent cut-off for serum ferritin ($<12.0 \mu\text{g/L}$) to define iron deficiency, whereas we used serum ferritin $<15 \mu\text{g/L}$, the level suggested by the World Health Organization (WHO).

The present study also found significantly higher haemoglobin and serum ferritin concentrations and lower prevalence of anaemia, iron deficiency and IDA in pregnant women in high groundwater iron areas than in the pregnant women in low groundwater iron areas. A recent publication based on the NMS 2011–2012 also reported a similar difference in serum ferritin concentrations in NPNL women living in low and high groundwater iron areas but found no difference in prevalence of iron deficiency [12].

The British Geological Survey conducted a study on mineral content in groundwater and found variable amounts of dissolved iron in groundwater of Bangladesh, ranging from a trace to very high concentrations. [9]. Another study, in rural areas of the northwestern districts of Gaibandha and Rangpur in Bangladesh, also reported wide difference (25-fold) in the groundwater iron concentration between tube wells less than 50 m apart [10]. In the present study, we also found a wide variation in iron concentration in tube-well water. In low groundwater iron areas, iron concentrations in tube-well water used by three-quarters of the participants were below 1.0 mg/L , 13.3% were between 2 and 10 mg/L , and none was $\geq 10 \text{ mg/L}$. In high groundwater iron areas, iron concentrations in tube-well water used by 45.5% of the participants were below 1.0 mg/L , 28.3% were 2– 10 mg/L and 14.9% were 10.0 mg/L or above (data not shown). Further, it is important to recognize that the bioavailability and

amount of iron absorbed from the groundwater depend on form and amount of iron consumed [18]. Studies have shown that iron in the groundwater of Bangladesh is predominantly ferrous (Fe^{2+}) iron, which is more bioavailable than the ferric (Fe^{3+}) form [19-20]. However, many households in Bangladesh have a practice of keeping water for a considerable time after collection. On exposure to air, the ferrous form of iron undergoes oxidation to insoluble ferric form and precipitates out [21]. Eighty-one percent of the participants were used to collecting water in the drinking vessel and drinking within minutes after collection, and the rest had a habit of drinking after dissolved minerals had precipitated. We measured iron concentration in water at the stage of consumption to estimate daily iron intake from drinking water. Intake values were divided into quartiles, and prevalence of anaemia, iron deficiency and IDA were compared by quartiles. Although there was no difference in the prevalence of anaemia, the prevalence of both iron deficiency and IDA in the third and fourth quartiles of daily iron intake groups were significantly lower than in the lowest quartile of daily iron intake group.

Further, we did a logistic regression to determine the independent association of daily iron intake from drinking water with iron deficiency, with adjustment for potential confounders. The results showed that the intake of iron from drinking water was inversely associated with risk of iron deficiency. Merrill et al. [7] also observed a strong, positive, dose-response association between iron intake from groundwater and iron status in non-pregnant women living in northern Bangladesh [7]. The findings of the present study corroborate the suggestion made by previous studies that iron in groundwater might play a role in the iron status of the Bangladeshi population [7, 12]. Contrary to Merrill et al.'s observation [7], we found an independent positive association between frequency of consumption of red meat and iron status. The logistic regression revealed that those participants who had red meat three times and more per week were less likely to develop iron deficiency. One possible explanation for the difference between the two studies could be the difference in iron content in the groundwater between the two studies. In our study, the median (interquartile range) daily iron intake from drinking water was 1.29 (0.41–5.80) mg, whereas it was 42 (16.0–71.0) mg their study. It is of note that although over the years, the consumption of animal-source foods has increased in the country [22], it constitutes only a small share of the total dietary iron [6]. We also did a separate logistic regression for anaemia. Although the

association between daily iron intake from drinking water and anaemia was not observed, gestational age and iron status were found to be significant predictors of anaemia.

In the present study, 38.7% of anaemic pregnant women had IDA. Further, a significantly higher proportion (50%) of anaemic pregnant women living in low groundwater iron areas had IDA than the anaemic pregnant women (25%) living in high groundwater iron areas. Thus, the prevalence of anaemia remains high among rural pregnant women, and 50–75% of anaemia, depending on the groundwater iron area, may not be attributable to iron deficiency. Anaemia has been found to be associated with increased maternal and perinatal death [23]. Furthermore, consequences of morbidity associated with chronic anaemia extend to loss of productivity from reduced work capacity, cognitive impairment and increased susceptibility to infections [24], which also imposes a substantial economic burden on an individual [25]. Among non-nutritional contributors to anaemia [malaria, worm infestation, chronic infections and genetic disorders (haemoglobinopathies)] [26–28], it is noted that the risk of malaria is low in Bangladesh. Although there is no national data on the prevalence of haemoglobinopathies, they were considered a likely contributor in one study conducted among women in rural northwestern Bangladesh that found a 28% prevalence of haemoglobinopathies [29]. However, a recent review of thalassemia in South Asia reported an estimated prevalence of beta-thalassemia in the range of 3–6% and 3–4% for Hb-E in Bangladesh [30]. In addition, deficiencies of other micronutrients such as vitamins A, C, B₂, B₆, B₁₂ and folic acid are known to play a role in the causation of anaemia [31]. Thus, the findings of the present study reinforce the need for further research to identify the other nutritional and non-nutritional causes of anaemia.

This study has some limitations. First, this study was conducted on a convenience sample; thus, the study findings may not be representative of the wider population, so the results should be interpreted with caution. Second, stratification of the data by daily iron intake from drinking water by quartile and to examine its association with anaemia and iron deficiency may not have adequate statistical power. However, the findings of this work are in line with previous reports. Thus, the potential is that the findings are very close to the actual situation.

This study shows a high prevalence of anaemia among rural Bangladeshi women in the early stage of pregnancy. Moreover, there is a differential prevalence of anaemia and iron deficiency in pregnant women living in areas of high and low iron in groundwater. Iron status was found to be independently associated with daily intake of iron from drinking water. Because less than 40% of anaemia could be accounted for by iron deficiency, further research to identify other nutritional and non-nutritional contributors to anaemia is needed to develop appropriate interventions to prevent and control anaemia among rural pregnant women in Bangladesh.

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Authors' contributions: F.A. took the lead in study planning and design, provided guidance on the data collection, and wrote the manuscript; M.R.K. supervised the blood collection and laboratory analysis; N.S. supervised the field work and data collection; K.M.U.A. and A.H. were responsible for water iron analysis; M.R.K. and I.A.C. contributed to the study design and manuscript writing. R.C. contributed to the statistical analysis of the data and its interpretation. F.A. had primary responsibility for final content. All authors read and approved the final manuscript.

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Figure 1: Prevalence of anaemia, iron deficiency anaemia and iron deficiency in pregnant women by daily iron intake from drinking water.

a,b differs significantly from each other ; x,y differs significantly from each other ; p,q differs significantly from each other

Table 1: Socio-demographic and pregnancy related characteristics of the participants, by groundwater iron area

Variable	All		High iron area		Low iron area		<i>p</i> -value
	n	%	n	%	n	%	
Age (year)							
13-19	126	24.1	63	24.0	63	24.2	0.105
20-24	166	31.8	73	27.9	93	35.8	
>25	230	44.1	126	48.1	104	40.0	
Parity							
Nulliparous	186	35.6	91	34.7	95	36.5	0.891
One	216	41.4	109	41.6	107	41.2	
Two or more	120	23.0	62	23.7	58	22.3	
Gestational age (week)							
≤12	71	13.6	38	14.5	33	12.7	0.546
>12	451	86.4	224	85.5	227	87.3	
Subject's education							
Functionally illiterate	230	44.1	99	37.8	131	50.4	0.010
Incomplete secondary	203	38.9	110	42.0	93	35.8	
Complete secondary and higher	89	17.0	53	20.2	36	13.8	
Husband's education							
Functionally illiterate	300	57.4	133	50.8	167	64.2	0.002
Incomplete secondary	111	21.3	58	22.1	53	20.4	
Complete secondary and higher	111	21.3	71	27.1	40	15.4	
Husband's occupation							
Unemployed/labourer	205	39.3	76	29.0	129	49.6	0.001
Farmer	136	26.0	79	30.2	57	21.9	
Business	108	20.7	63	24.0	45	17.3	
Service	73	14.0	44	16.8	29	11.2	
Subject's occupation							
No	502	96.2	250	95.4	252	96.9	0.371
Yes	20	3.8	12	4.6	8	3.1	
Household size							
Small (up to 4)	315	60.3	164	62.6	151	58.1	0.291
Large (> 4)	207	39.7	98	37.4	109	41.9	
Cultivable land ownership (acre)							
No land	295	56.5	136	51.9	159	61.1	

Small land holding	< 0.50	135	25.9	81	30.9	54	20.8	0.027
Sizable landholding	≥ 0.50	92	17.6	45	17.2	47	18.1	

Table 2: Mean haemoglobin and serum ferritin concentrations, prevalence of anaemia, iron deficiency, and iron deficiency anaemia among pregnant women, by groundwater iron area

	High iron area			Low iron area			
	n	Mean	95% CI	n	Mean	95% CI	<i>P</i> -value
Concentration							
Haemoglobin (g/dL)	262	11.43	11.31-11.54	260	11.25	11.13-11.36	0.033 [¶]
Serum ferritin* (µg/L)	262	25.49	23.16-28.03	260	19.75	17.95-21.74	0.001 ^{¶**}
Prevalence		%			%		
Anaemia (%)	79	30.2	24.7-36.1	102	39.2	33.3-45.5	0.029
Iron deficiency (%)	55	21.0	16.1-25.9	86	33.1	27.4-38.8	0.002
Iron deficiency anaemia (%)	20	7.6	4.4-10.9	50	19.2	14.4-24.0	0.0001

*Serum ferritin adjusted for elevated CRP ≥ 10.0 mg/l and AGP ≥ 1.0 mg/l. ** log transformed.

[†]Adjusted for subject's education, husband's education and occupation, cultivable land and gestational age.

Table 3: Daily intake of drinking water and iron from drinking water by the pregnant women, by groundwater iron area

Variable		All	High iron area	Low iron area
Drinking water (L/day)	Mean (SD)	2.52 (0.92)	2.77 (0.98)	2.24 (0.76)
	Median (IQR)	2.4 (1.8-3.2)	2.80 (2.0-3.4)	2.0 (1.6-2.6)
	Range	0.8-6.0	0.8-6.0	1.0-5.0
Iron concentration in drinking water (mg/L)	Mean (SD)	2.48 (4.49)	4.14 (5.76)	0.83 (1.35)
	Median (IQR)	0.55 (0.21-2.30)	1.28 (0.37-5.27)	0.31 (0.14-0.90)
	Range	0.00-28.60	0.00-28.60	0.00-9.05
Iron intake from drinking water (mg/day)	Mean (SD)	6.64 (12.4)	11.11 (15.69)	1.77 (2.96)
	Median (IQR)	1.29 (0.41-5.8)	3.62 (0.91-15.15)	0.61 (0.26-1.82)
	Range	0.00-79.45	0.00-79.45	0.00-21.72

Table 4: Logistic regression analysis (odds ratios) for iron deficiency and anaemia among pregnant women.

	Variable	OR	95% CI	
Iron deficiency	Age (year)			
	≥25 y (reference)			
	13-19 y	1.69	0.90	3.17
	20-24 y	1.70	1.26	2.29
	Subject's education			
	Functionally illiterate (reference)			
	Incomplete secondary	0.94	0.74	1.18
	Secondary and higher	2.15	1.08	4.30
	Gestational age (week)			
	≤ 12 (reference)			
	>12	3.48	1.16	10.41
	Intake of red meat (times/week)			
	0-<3 (reference)			
	≥3	0.62	0.42	0.90
	Daily iron intake from drinking water			
	Lowest quartile (reference)			
	2 nd quartile	0.84	0.69	1.01
	3 rd quartile	0.41	0.27	0.61
	Highest quartile	0.29	0.25	0.34
Anaemia	Husband's occupation			
	Unemployed/daily labourer (reference)			
	Business	0.88	0.55	1.40
	Farmer	0.54	0.48	0.61
	Service	0.77	0.44	1.36
	Gestational age (week)			
	≤ 12 (reference)			
	>12	2.69	1.42	5.07
	Iron status			
	Normal (serum ferritin ≥15.0 µg/L) (reference)			
	Deficient (serum ferritin <15.0 µg/L)	2.35	1.56	3.54
	Daily iron intake from drinking water			
	Lowest quartile (reference)			
	2 nd quartile	0.51	0.26	0.97
	3 rd quartile	0.79	0.54	1.16
	Highest quartile	0.86	0.48	1.56

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Author: Faruk Ahmed, Moududur Rahman Khan, Najma Shaheen, Kazi Matin Uddin Ahmed, Aziz Hasan, Ireen Akhtar Chowdhury, Rafiqul Chowdhury

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