Iron Status of Women Is Associated with the Iron Concentration of Potable Groundwater in Rural Bangladesh1–3

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Abstract

Women of reproductive age are at a high risk of iron deficiency, often as a result of diets low in bioavailable iron. In some settings, the iron content of domestic groundwater sources is high, yet its contribution to iron intake and status has not been examined. In a rural Bangladeshi population of women deficient in dietary iron, we evaluated the association between groundwater iron intake and iron status. In 2008, participants (n = 209 with complete data) were visited to collect data on 7-d food frequency, 7-d morbidity history, 24-h drinking water intake, and rice preparation, and to measure the groundwater iron concentration. Blood was collected to assess iron and infection status. Plasma ferritin (μg/L) and body iron (mg/kg) concentrations were [median (IQR)] 67 (46, 99) and 10.4 ± 2.6, respectively, and the prevalence of iron deficiency (ferritin < 12 μg/L) was 0%. Daily iron intake from water [42 mg (18, 71)] was positively correlated with plasma ferritin (r = 0.36) and total body iron (r = 0.35) (P < 0.001 for both). In adjusted linear regression analyses, plasma ferritin increased by 6.1% (95% CI: 3.8, 8.4%) and body iron by 0.3 mg/kg (0.2, 0.4) for every 10-mg increase in iron intake from water (P < 0.001). In this rural area of northern Bangladesh, women of reproductive age had no iron deficiency likely attributable to iron consumed from drinking groundwater, which contributed substantially to dietary intake. These findings suggest that iron intake from water should be included in dietary assessments in such settings. J. Nutr. 141: 944–949, 2011.

Introduction

Populations in resource-poor settings who subsist on cereal-based diets low in bioavailable iron and rarely consume supplements risk having iron deficiency, estimated to be the world’s most common single micronutrient deficiency and cause of anemia (1,2). Women of reproductive age experience a disproportionately higher prevalence due to the increased demand for iron associated with menstruation and pregnancy (3). Due to the expected high prevalence of iron deficiency in developing countries and its possible health consequences, which include decreased work capacity and poor reproductive outcomes (4), much attention has been focusing on developing effective interventions, such as supplementation and food fortification, to reduce the risk (5,6).

When calculated in different settings within Bangladesh, iron deficiency anemia was prevalent in 2, 11, and 29% of women in populations with 28, 37, and 48%, anemia, respectively (7–9). These surveys suggest that iron deficiency rates and contribution to the prevalence of anemia in the country may not be as uniform and common as projected.

Previous data have revealed elevated and variable levels of dissolved iron in groundwater, used by over 90% of the population for domestic purposes (10), across Bangladesh (11). One of the earliest studies exploring the association between iron in water and nutritional status showed that groundwater iron concentration was positively associated with linear growth of children in Bangladesh (12). Additionally, multiple studies in Brazilian daycare centers and households have found that iron status is improved and the prevalence of anemia and iron deficiency anemia is reduced among children and adults consuming iron-fortified water (up to 30 mg/L elemental iron) (13–17).

Based on this evidence, fortifying water with iron has been suggested as a simple, cheap, and effective alternative to other food fortification methods (18).

Therefore, we recently assessed the concentration of iron in the groundwater (19) and estimated, among women, the median daily iron intake from drinking groundwater to be 41 mg (20). We conducted the current study to investigate the potential influence on iron status of consuming iron from a natural groundwater source in...
Materials and Methods

The aim of this study was to investigate the role of iron in drinking water among normal, nonpregnant women of reproductive age living in northern Bangladesh whose identity and residence were known through past participation in a large, community-based antenatal nutrition research project (JiVitA Project) that was implemented in an area of ~435 km² in the District of Gaibandha between 2001 and 2007 (JiVitA-1) (21). Details of this follow-up study, conducted in 2008, have been published elsewhere (20). In brief, the present study was limited to a purposively selected, mapped area of 32 of 596 community clusters comprising a substudy area of 24 km² with a population of ~45,000, nested within the larger, rural JiVitA Project area. Within this set of communities, 321 women were eligible based on fully participating and contributing a term pregnancy with a live-born infant to the earlier trial, maintaining their original residence since that time, and not having been pregnant at the time of enrollment in the present study (Supplemental Fig. 1). Pregnancy at enrollment was an excluded condition due to the known influence of pregnancy on iron status and the planned blood sample collection 4–6 mo later in round 2 (3). Pregnancy status at the time of blood sample collection was recorded.

Consenting participants were surveyed during 2 rounds in 2 different seasons in 2008, the first round being conducted from May to July (dry/early monsoon season) and the second round from October to November (postmonsoon). During both rounds, local, trained, female project staff conducted home interviews and collected and analyzed groundwater from participant-identified tubewells for iron content in addition to doing anthropometry at round 1 and phlebotomy at round 2.

Interviews. During each interview, participants completed a 7-d, 42-item FFQ, a 7-d habitual recall of tea and coffee consumption within 30 min of a meal, and a 30-d recall of nutrient supplement use. Participants were also asked detailed questions about their rice preparation, including cooking pot material, and consumption and drinking water consumption in the previous 24 h using a semiquantitative recall aided by 6 meal and between-mealtime prompts: with the morning meal, between the morning meal and lunch, with lunch, between lunch and dinner, and after dinner and before the morning meal. The unit of measurement for the drinking water recall was each participant’s personal drinking container, the volume of which was measured to within 10 mL in the field during the interview using a calibrated 500-mL measuring cup.

Participants identified their predominant drinking water source and were probed about their perception of iron level in the water based on a 4-point scale (none, a little, a medium amount, and a lot). This question was asked to explore if a simple, replicable question could be used to gauge iron concentration in a domestic water source. If the drinking water source was a tubewell, a plastic pipe with a unique 5-digit identification number was riveted to the structure. All labeled tubewells were analyzed in each round for total iron concentration [≥0.1 mg/L, FerroVer method, HACCP Iron Test kit, Model IR-18B, previously validated (22)] following methods described in detail elsewhere (20).

At both visits, participants also completed a 7-d history of high fever, vomiting, painful urination, dysentery, and diarrhea. During round 1, an interim pregnancy history was obtained and socioeconomic status was updated using abbreviated versions of the original interview modules.

Anthropometry. During round 1, standard anthropometric procedures (23) were used to measure weight (±0.1 kg) on a digital Uniscale (UNICEF/SECA, Gmbh & Co, calibrated daily), height (±0.1 cm) using a portable stadiometer consisting of a metal footplate, affixed metal tape with carpenter’s level (produced by the JiVitA Project, Gaibandha, Bangladesh), and mid-upper arm circumference (MUAC; ± 0.1 cm) using a nonstretch insertion tape (produced by the JiVitA Project) patterned on the original Zerfas design (24). The median of triplicate measures was used for height and MUAC.

Iron status. Capillary blood (nonfasting, 300 μL, EDTA coated, Safe-T-Fill, RAM Scientific) was collected in round 2. The hemoglobin (Hb) concentration was assessed on the spot at the time of blood sample collection with a B-Hemoglobin photometer (HemoCue). HemoCue instruments were used within 10 d of opening the bottle, which was stored at ~4°C overnight. Samples were immediately placed into insulated cool bags until same-day processing at our project laboratory in Gaibandha. Plasma aliquots were stored in liquid nitrogen until analysis of ferritin (μg/L), soluble transferrin receptor (TR; mg/L), and C-reactive protein (CRP; mg/L) at the International Centre for Diarrheal Disease Research, Bangladesh in Dhaka. These proteins were analyzed using a sandwich ELISA method described elsewhere by Erhardt et al. (25) using Roche standards (Roche Diagnostics) to obtain calibration curves. Precision was checked with pooled samples and accuracy was checked with manufacturer-supplied controls. The inter-assay CV were <5% for all checks except the TR control with a mean concentration of 6.34 mg/L and CV of 7.1% and the CRP pooled sample with a value range of 0.5–0.8 mg/L and CV of 16.3%.

Statistical analysis. From an original sample of 400 women listed from our earlier field trial, 321 were resident and not pregnant at enrollment in 2008 and thus eligible for this study, of whom 14 (4%) refused participation, resulting in 307 consenting participants enrolled at the outset (Supplemental Fig. 1). Of those, complete data were available for 209 women, all of whom were included in these analyses. Eight participants were in their first trimester of pregnancy in round 2.

Anthropometric distributions were dichotomized to classify wasting malnutrition using BMI <18.5 kg/m² (26) and MUAC <22.0 cm (27). Dietary data were categorized into 5 food groups: red meat (meat and liver), chicken and fish, dark green leafy vegetables (DGLV), fruits (tomato, potato, guava, orange, papaya, pineapple, and lime), and dairy and eggs. For each food group, individual mean 7-d intake frequency of rounds 1 and 2 was used to represent usual consumption and was grouped into binary variables (≥2 vs. < 3 times/wk). Iron intake through groundwater (mg/d) was analyzed as a continuous and categorical variable based on common supplementary iron dosages: 0–30 mg/d, 31–60, and >60 (28). Reported iron supplement use was dichotomized as having been consumed fewer than 15 of the past 30 d.

One study has shown that intra-subject ferritin concentration may vary between capillary compared with venous blood samples. To facilitate comparability with other studies using venous blood, 2 adjustments were applied to the capillary ferritin blood values in this study: −5.2% for values from 0 to 200 μg/L (29) and −8.3% for all values (30). To calculate body iron using the Cook et al. (31) formula: body iron (mg/kg) = [1 − ([log10 TR(W)/ferritin (μg/L)] − 2.8229)/0.1207]), TR values were converted with the formula 1.5 × TR (mg/L) + 0.35 (32) from the International Centre for Diarrheal Disease Research, Bangladesh results based on Roche calibration curves to those that reflected the in-house ELISA developed by Flowers et al. (33) and used to develop the body iron equation. Biomarker cutoffs were applied to classify the following abnormal states: iron deficiency, plasma ferritin <12 μg/L, TR > 8.5 mg/L, or body iron ≤ 0.0 mg/kg (1 mg Fe = 55.8 mmol); anemia, Hb < 120 g/L and 110 g/L for nonpregnant and pregnant women, respectively (34); iron deficiency anemia, the presence of anemia and iron deficiency; risk of iron overload, plasma ferritin >150 μg/L (35); and infection, CRP > 5.0 mg/L (36).

Using a Z-test for proportions, Student’s t and ANOVA tests for continuous distributions, and Fisher’s exact test for multiple category variables, anthropometric, demographic, and socioeconomic status variables were compared between participants with and without complete data. Additionally, the distributions of plasma ferritin, which was log transformed (to the base e) to achieve normality, and body iron were compared by presence of subclinical infection, recent morbidity, dietary intake frequency, and estimated levels of daily iron intake through drinking water. Data are presented as a mean ± SD or median (IQR) unless otherwise stated.
Linear regression was used to evaluate relative influence of demographic, anthropometric, dietary, and morbidity factors on iron status [log plasma ferritin (ln μg/L) and body iron (mg/kg)]. Results of plasma ferritin were reported as percent change calculated using the formula \( e^{\beta_{regression \ coefficient} \cdot 100 – 100} \). Covariates with a potential influence on the outcome, \( P < 0.10 \) in univariate analyses, were retained for final model development. For each outcome, a best fit model was selected using forward stepwise regression and log likelihood ratio tests. One participant with extreme BMI, MUAC, CRP, and plasma ferritin was excluded as a regression outlier based on regression diagnostics and sensitivity analyses.

All analyses were performed using STATA statistical software package version 11.0 (37). This study and the original JIVitA-1 trial were approved by the Bangladesh Medical Research Council, Dhaka, Bangladesh and the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

**Results**

Baseline characteristics did not differ between enrolled participants by availability of complete data (Table 1). For those included in all analyses \( (n = 209) \), 65% of the women were between 20 and 30 y of age and 82% had more than 1 child. The median number of years of education was 7 (4,9) for the 62% \( (n = 130) \) reporting any formal education. BMI (kg/m²) and MUAC (cm) were 19.3 ± 2.5 and 23.8 ± 2.3, respectively.

Iron and infection status indicators are summarized in Table 2. No participants had iron deficiency anemia. Adjusting plasma ferritin to account for the potential differential effects of a capillary compared with a venous blood draw did not change the prevalence, with a plasma ferritin concentration < 12 μg/L. Plasma ferritin and calculated total body iron were not associated with wasting undernutrition \[ \text{Plasma ferritin} \text{ and calculated total body iron were not associated} \]. Iron status indicators also did not differ by pregnancy status at the time of assessment for the 8 participants in their first trimester at the time of blood collection.

**TABLE 1** General participant characteristics at enrollment \( (n = 209) \)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>( n(%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>43 (21)</td>
</tr>
<tr>
<td>20–24</td>
<td>81 (39)</td>
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<tr>
<td>25–29</td>
<td>54 (26)</td>
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<tr>
<td>≥30</td>
<td>31 (15)</td>
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<td>Parity</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37 (18)</td>
</tr>
<tr>
<td>2 or 3</td>
<td>132 (63)</td>
</tr>
<tr>
<td>≥4</td>
<td>40 (19)</td>
</tr>
<tr>
<td>Education, y</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>79 (38)</td>
</tr>
<tr>
<td>1–9</td>
<td>110 (53)</td>
</tr>
<tr>
<td>≥10</td>
<td>20 (10)</td>
</tr>
<tr>
<td>Wealth index, (^1) quartile</td>
<td></td>
</tr>
<tr>
<td>Lowest</td>
<td>51 (24)</td>
</tr>
<tr>
<td>Highest</td>
<td>48 (23)</td>
</tr>
<tr>
<td>Religion (% Muslim)</td>
<td>187 (89)</td>
</tr>
<tr>
<td>Anthropometry(^2)</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>43.6 ± 6.4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>150.2 ± 5.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.8 ± 2.5</td>
</tr>
</tbody>
</table>

\(^1\) Wealth index defined for entire JIVitA-1 population based on JIVitA-1 enrollment data collected 2–5 y prior.

Recent morbidity was not associated with elevated CRP. In contrast to plasma ferritin, which did not differ by subclinical infection status \( (P = 0.08) \), calculated total body iron was higher among those with elevated CRP \( (11.9 ± 1.5 \text{ mg/kg}) \) than those without \( (10.3 ± 2.6 \text{ mg/kg}) \) \( (P = 0.03) \). Seven percent \( (n = 14) \) were at risk of iron overload, only 1 of whom showed signs of elevated CRP.

Nearly all participants reported consuming red meat \( (96%, n = 200) \) and DGLV \( (76%, n = 159) < 3 \text{ times/wk} \). In contrast, the majority of women reported consuming fish or chicken \( (86%, n = 180) < 3 \text{ times/wk} \). Roughly one-half \( (n = 108) \) consumed fruits often. Plasma ferritin was higher among those who consumed DGLV frequently \[ [79 (52, 116)] \) than infrequently \[ [64 (42, 94)] \) \( (P = 0.05) \) but did not differ for other food groups (Fig. 1). Participants usually consumed 42 mg/d \( (18, 71) \) range: 0–151) of iron through drinking groundwater alone.

Fewer than 2% of participants consumed tea within 30 min of a meal. Coffee consumption was nonexistent. Regular nutrient supplement use was rare \[ [n = 4 (2%) \text{ and } 10 (5%) \text{ in rounds 1 and 2, respectively}] \) and was not associated with any indicator of iron status. At both rounds, participants predominantly used aluminum \( (40%) \) or clay \( (36%) \), but never iron, cooking pots for rice.

Iron status was positively associated with iron intake from drinking groundwater in crude and adjusted analyses (Table 3). Iron status also increased by ground iron intake when defined by levels similar to supplemental iron doses \[ \text{plasma ferritin} (\mu g/L) \) for 0–30 mg/d, 31–60, and >60 \( [n = 77, 67, 65]: 55 (36, 83), 66 (49, 99), 83 (60, 119), \text{ respectively, and body iron} (\text{mg/kg}): 7.6 ± 2.5, 8.6 ± 2.3, 9.7 ± 2.3, \text{ respectively} \) \( (P < 0.001) \). Interestingly, groundwater iron intake was positively associated with participants’ perception of iron level in the groundwater (Fig. 2). Correspondingly, plasma ferritin increased with perceived iron level in groundwater \[ \text{plasma ferritin} (\mu g/L) \) for none, a little, a medium amount, and a lot, \( n = 30, 49, 49, \text{ and } 78, \text{ respectively, excluding those who filtered groundwater: 52 (24, 82), 57 (42, 84), 69 (39, 110), 79 (55, 106), \text{ P < 0.001}} \).

**Discussion**

We have shown that daily iron intake from groundwater in a rural area of northwestern Bangladesh, which was >42 mg for one-half the population of women, was positively related to plasma ferritin and total body iron. Although we observed minimal evidence of iron overload, our findings reveal a strong, positive, dose-response association between natural iron content in groundwater, intake of iron from such sources, and iron status of women, such that body stores would be expected to be 0.3 mg/kg higher for every 10-mg increment in daily iron intake from water. Plasma ferritin was estimated to be ~6% higher for the same increment, raising the prospect that local water consumption exerts a strong and positive effect on iron status in this rural population, raising iron nutriture and virtually eliminating iron deficiency without posing risks associated with iron excess.

The current investigation was prompted by an earlier observation that virtually all women living in a circumscribed region of northern Bangladesh who had been participating in a vitamin A field trial were iron sufficient during pregnancy and lactation (38), based on the fact that concentrations of plasma ferritin, a conventional indicator of iron status, were >12 μg/L. Anemia, on the other hand, affected 22–41% of the women during pregnancy (K. P. West, Jr, unpublished data). This atypical pattern suggested that iron deficiency was not a public health problem and that anemia was due to other causes. Usual diet,
types of morbidity, lifestyles, and external environment were typical for the region, suggesting exposures other than those usually considered may be responsible for their iron sufficiency.

Study women consumed a typical Bangladeshi diet characterized by high rice consumption and relatively infrequent intakes of heme-rich red meats but frequent fish intake. This dietary pattern has been shown elsewhere to provide an iron intake of $6.9 \pm 3.0 \text{ mg/d (mean} \pm \text{ SD), 12\% of which would be bioavailable assuming body iron stores of 250 \text{ mg (39)}, an insufficient amount to maintain adequate iron status. However, in Gaibandha, we observed that one-half of studied female residents consumed $\geq 42 \text{ mg of iron from their drinking water. This amount approaches the tolerable upper intake level of } 60 \text{ mg/L in water}. The score was positively associated with both iron status and intake frequencies of multiple food groups, using a 42-item, 7-d, repeated FFQ, including likely iron-deficient foods (52). We think that these results provide strong support for the capability of water iron intake, if sufficiently high, to maintain a normal iron status in a community, suggesting that iron deficiency is not ubiquitous in Bangladesh. Of importance, elevated levels of groundwater iron are not unique to this study area. A British Geologic Survey of the mineral content in groundwater across Bangladesh showed that iron concentration is elevated in many parts of the country, reaching $>60 \text{ mg/L in some areas (11). Additionally, that survey and others have shown that the aquifer environment is reducing (19,49), meaning that dissolved iron is predominantly ferrous (Fe^{2+}), a form that is readily absorbed in the gut (50). Furthermore, the WHO has described that “minerals in water are subject to most of the same determinants of bioavailability that affect the utilization of those minerals in foods (51).” This statement is supported by findings from water with electrolytically reduced iron shown to be readily absorbed (52).

When asked, participants in our study were able to independently and accurately perceive iron content in their tubewell water source, using a 4-point scale. Their ability to do so, highlighted in a previous paper (20), is most likely the result of organoleptic differences associated with changes in the oxidation state of iron in the water. The score was positively associated with both iron status and intake frequencies of multiple food groups.
estimated daily iron intake through groundwater and iron status, suggesting that a simple question about the amount of iron thought to be in pumped water from a usual well could be used to enhance community dietary surveys to assess exposure to water-based iron and its influence on status.

A limitation of the study was the reduction in the number of participants used in analyses from the number who participated in both data collection rounds. Although blood sample collection refusal rates were low (5%), the main reason for incomplete data was an unexpectedly high rate of insufficient blood samples resulting from logistical and technical challenges encountered under remote field conditions. Notably, participants with and without hematological data were comparable in population characteristics, suggesting a representative subset of participants was studied.

In this study, we have not examined the second observation in this population, that of anemia being highly prevalent despite apparent iron sufficiency. This will be addressed in a subsequent report. However, an inference derived from the current analysis is that universal iron supplementation of women of reproductive age, as well as presumptive treatment of anemia with iron supplements, recommended as standard of care where the prevalence of anemia is >20–30% (53), is likely to be ineffective in reducing anemia in this and similar environmental settings. On the contrary, regular and especially high iron supplement regimens may increase the risk of adverse health effects associated with chronic excess iron intake (54), reinforcing the premise that causes of anemia should be known and such data used to guide anemia treatment and prevention.

In conclusion, this study found that iron consumed through drinking groundwater is positively associated with iron status and a likely major factor in preventing iron deficiency where diets are otherwise low in bioavailable iron. Iron intake through water should be considered when evaluating dietary risks associated with iron deficiency.

Acknowledgments
R.D.M. and A.A.S designed the research; R.D.M., A.A.S., N.J., H.A., and A.B.L. conducted the research; K.S. provided essential materials; R.D.M., P.C., and K.P.W. Jr analyzed the data and wrote the paper; and R.D.M. had primary responsibility for the final content. All authors read and approved the final manuscript.

Literature Cited


