Determinants of iron status and Hb in the Bangladesh population: the role of groundwater iron

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Abstract

Objective: Using data from the national micronutrients survey 2011–2012, the present study explored the determinants of Fe status and Hb levels in Bangladesh with a particular focus on groundwater Fe.
Design: Cross-sectional study conducted at the nationwide scale.
Settings: The survey was conducted in 150 clusters, fifty in each of the three strata of rural, urban and slum.
Subjects: Three population groups: pre-school age children (6–59 months; PSAC), school age children (6–14 years; SAC) and non-pregnant non-lactating women (15–49 years; NPNLW).
Results: National prevalence of Fe deficiency was 10·7 %, 7·1 % and 3·9–9·5 % in PSAC, NPNLW and SAC, respectively. Prevalence of anaemia was 33·1 % (PSAC), 26·0 % (NPNLW) and 17·1–19·1 % (SAC). Multivariate regression analyses showed that the area with 'predominantly high groundwater Fe' was a determinant of higher serum ferritin levels in NPNLW (standardized $\beta = 0·19$; $P = 0·03$), SAC (standardized $\beta = 0·22$; $P = 0·01$) and PSAC (standardized $\beta = 0·20$; $P = 0·03$). This area also determined higher levels of Hb in PSAC (standardized $\beta = 0·14$; $P = 0·01$).
Conclusions: National prevalence of Fe deficiency in Bangladesh is low, contrary to the widely held assumption. High Fe level in groundwater is associated with higher Fe status (all populations) and higher Hb level (PSAC).

Anaemia is one of the largest public health problems in Bangladesh. In 2011, the national prevalence of anaemia was 51 % in children under 5 years of age and 42 % among non-pregnant women[1]. The causes of anaemia in populations are inadequate intake of micronutrients, poor dietary diversity, infection, haemoglobinopathy and household food insecurity[2]. Among the micronutrients, Fe deficiency is assumed to be a major reason for anaemia. However, a recent study conducted in a north-western district of Bangladesh revealed 0 % prevalence of subclinical Fe deficiency in women of reproductive age[3]. The study attributed this very low prevalence of Fe deficiency to drinking groundwater from tube wells that were a rich source of naturally occurring Fe. In addition, a few other studies have reported a low prevalence of Fe deficiency of 8 %, 13 % and 17 % among pregnant women,

married nulliparous women and schoolchildren, respectively[4–6]. The present paper aimed to explore the association of groundwater Fe with population-level Fe nutrition and Hb status, at a nationwide scale. This is essentially an assessment of the determinants of Fe and Hb status along with studying the role of groundwater Fe in explaining the associations. The data analysed for the present paper were taken from the national micronutrient survey of Bangladesh conducted in 2011–2012.

Methods

Sampling and study population

A multistage random sampling procedure was applied to select the study participants from rural, urban and slum

strata spread all over Bangladesh. In the first stage, 150 clusters (fifty clusters in each stratum) from the 15 000 clusters of the Bangladesh Multiple Indicator Cluster Survey (MICS) 2009 sampling frame were randomly selected. In the second stage a segment of fifty households was randomly chosen from each of the selected clusters. In the third stage twenty households were selected randomly from the fifty-household segment; those twenty households formed the definitive sampling frame from which the required number of study participants was selected.

The sample size was calculated to estimate the parameters for each stratum with defined precisions (see online supplementary material, Supplemental Table 1). The national prevalence of Fe deficiency and anaemia was estimated by combining all three strata together through the application of sampling weights. Data were collected in three population groups: pre-school age children (PSAC; 6–59 months), school age children (SAC; 6–14 years) and non-pregnant non-lactating women (NPNLW; 15–49 years).

Data and biological parameters

Data on socio-economic status (SES), household possession of assets, house construction material, household food insecurity, morbidity of children and food intake of the participants were analysed. SES was assessed by constructing the wealth index[7]. We referred to the British Geological Survey of 2001 to report the levels of Fe in groundwater[8]. The smoothed map prepared by the 2001 British Geological Survey depicted areas with differential Fe concentration using predominantly two groups of colour shades: shades of blue and shades of red (Fig. 1). The spectrum of colours, from deep blue to light blue to green to light red to deep red, corresponded to increasing level of Fe in water. Groundwater Fe concentration >2·0 mg/l is considered 'high' depending on the per litre equivalent of the Institute of Medicine's recommended tolerable upper intake level of 45 mg for daily Fe intake in adults (excluding Fe supplements) assuming water consumption of 2 litres/d[9,10]. In the map we demarcated the areas with low Fe in water using shades of blue. However, according to the map, some small patches with lighter shades of blue are areas that have Fe concentration of 2·1–2·8 mg/l. Hence some degree of misclassification was apparent. In addressing this we considered the Fe concentration of 2·8 mg/l as a cut-off, and termed the areas falling below that level as areas with predominantly low groundwater Fe (PLGWI) and designated areas with Fe concentration >2·8 mg/l (i.e. the different shades of red and some patchy areas of green) as areas with predominantly high groundwater Fe (PHGWI).

Household food insecurity was assessed by the Household Food Insecurity Access Scale (HFIAS)[11]. The HFIAS tool was pre-tested and customized to fit the
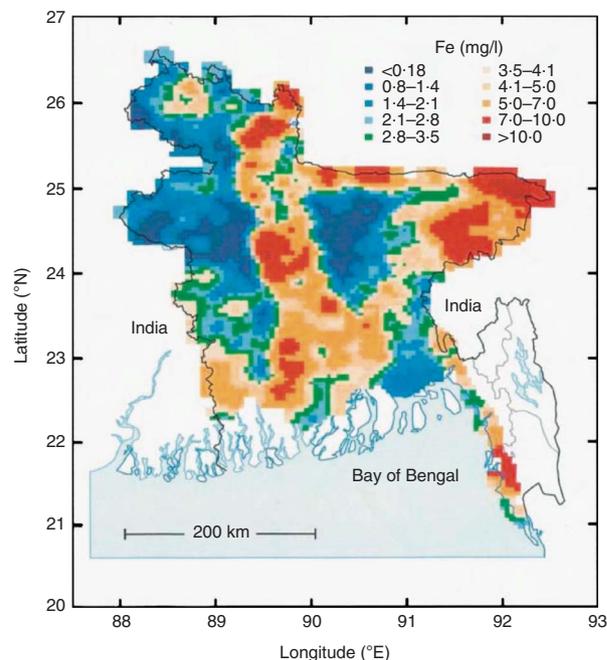
Fig. 1 (colour online) Iron concentration in groundwater in Bangladesh: British Geological Survey 2001[8]. According to the 2001 British Geological Survey, either side of the Brahmaputra basin, south of the Ganges basin and north-eastern haor regions (low plains with water mass) are predominantly high groundwater iron areas (denoted by shades of red), while the Barind and Madhupur tracts are the areas (denoted by shades of blue) with predominantly low iron in groundwater

Bangladeshi socio-economic and cultural contexts. To assess food consumption, a 7 d semi-quantitative FFQ was used taking into consideration commonly consumed Bangladeshi foods with a focus on Fe-rich foods[12]. Food photographs indicating the serving number and amount (grams) were used to assess the consumption. Raw food weight was calculated by using appropriate conversion factors[13]. Nutrient values were calculated per 100 g of raw food consumed using an updated food composition table on Bangladeshi foods[14]. Serum levels of the parameters ferritin, retinol, Hb, Zn, folate and vitamin B_{12} were assessed. The sample number for ferritin was less than that for Hb (Tables 1 and 2); the reasons were inadequate serum in aliquot, haemolysis and labelling error in sample identification by the field staff.

Biochemical analyses

Serum ferritin, C-reactive protein (CRP) and $α_1$-acid glycoprotein (AGP) were analysed by sandwich ELISA. Serum retinol was assessed by HPLC. Hb was assessed by using a HemoCue® Hb 301 system on venous blood. Serum Zn was assayed by atomic absorption spectrophotometry. Serum folate and vitamin B_{12} were analysed by electrochemiluminescence immunoassay on a Roche Diagnostics Cobas® 6000 analyser.

Adjusting for infection

Serum ferritin was adjusted for infection by estimating biomarkers of infection: CRP and AGP. The adjustment of serum ferritin for elevated CRP ($>10{\cdot}0$ mg/l)[15–17] and AGP ($>1{\cdot}0$ g/l) was done by calculating the correction factors following the methods described by Thurnham *et al.*[18] and Engle-Stone *et al.*[19]. Similarly correction factors for serum retinol and Zn were calculated and used to adjust the retinol and Zn levels in serum.

Quality control

All biochemical analyses were done in the nutritional biochemistry laboratory of icddr,b, Dhaka, Bangladesh. To control the quality of laboratory analyses, precinorm protein and precipath protein (Roche Diagnostics GmbH, Mannheim, Germany) were used as quality controls to check the accuracy and precision of ferritin, CRP and AGP assays. Quality control material was run in every lot and CV were calculated from the cumulative mean and standard deviation. The CV for ferritin, CRP and AGP was $3{\cdot}7$ %, $3{\cdot}9$ % and $5{\cdot}9$ %, respectively. Pooled serum quality control material was used for retinol and Zn (Bi-level serum toxicology control; UTAK Laboratories, Inc., Valencia, CA, USA) against the standard reference material; and CV were $1{\cdot}5$ % and $4{\cdot}0$ %, respectively. External quality assurance was conducted with two schemes: VITAL-EQA (Vitamin A Laboratory – External Quality Assurance; Centers for Disease Control and Prevention, Atlanta, GA, USA) for ferritin and CRP; and MMQAP (Micronutrients Measurement Quality Assurance Program; National Institute of Standards and Technology, Gaithersburg, MD, USA) for retinol.

Statistical analysis

Data analysis was done using the statistical software package STATA $10{\cdot}0$ SE. The key proportion estimates were calculated with a 95 % confidence interval. The key mean estimates were calculated with standard deviation through one-way ANOVA. Pearson's χ^2 test was used to compare proportions. Projected estimates of the population in the three strata (rural, urban and slum) in 2011 were $122{\cdot}6$, $23{\cdot}3$ and $5{\cdot}5$ million, respectively[20,21]. Despite the large difference in size, we selected fifty primary sampling units from each stratum, which resulted in differential selection probabilities and representations across the strata. Sampling weights were applied to the households in each stratum to compensate for the differential representations and to derive weighted national estimates combining the estimates of all the strata. Logarithmic transformation was carried out on the variables showing skewed distributions to convert them into a normal distribution. However, serum ferritin levels, which had a skewed distribution, were presented using medians and quartiles (online supplementary material, Supplemental Table 2). Statistical analyses such as proportion estimates, one-way ANOVA and multivariate linear regression analyses were done on weighted data. All analyses, including the regression models, were adjusted for clustering of data at the unit of the randomization level (primary sampling unit) using the cluster option in STATA. All predictor variables that were presumed to have an association with ferritin or Hb were entered in the univariate linear regression or bivariate analysis according to the type of the predictor variable. For example, univariate linear regression was performed for continuous predictors, such as age, household expenditure, dietary Fe intake, parity in women and serum status of micronutrients, to assess their association with ferritin or Hb. On the other hand, one-way ANOVA was performed for the categorical predictors, such as area of residence, wealth and household food insecurity categories. In both types of analyses, if there was a significant association with ferritin or Hb at $P<0{\cdot}05$ and it had the desired sign mark (i.e. plus or minus sign of the coefficient as per the logical expectation), the variable was selected for the multivariate regression[22]. The xi command in STATA was used to enable inclusion of categorical covariates in the multivariate models. Standardized β (effect size) was calculated to estimate the standardized change in outcomes (serum ferritin and Hb). Interaction analyses were done taking into consideration the variables that logically appear to be interacting in determining serum ferritin or Hb status. The predictor variables that were assumed to have interaction were entered in the initial regression model as an interaction term along with the interacting predictors. If the regression coefficient for the interaction term was non-significant ($P \geq 0{\cdot}05$) it was omitted; however, in case that the interaction term was statistically significant ($P<0{\cdot}05$) it was retained and entered into the final multivariate model[23]. In the case of serum ferritin the following interaction terms were assessed: (Hb × occupation), (occupation × stratum), (wealth index × stratum), (household food insecurity × stratum), (Hb × groundwater Fe), (groundwater Fe × stratum), (groundwater Fe × household food insecurity) and (groundwater Fe × wealth index). For Hb the interaction terms assessed were the following: (serum ferritin × serum retinol), (serum ferritin × serum Zn), (serum retinol × serum Zn), (serum ferritin × serum folate) and (groundwater Fe × stratum).

The Cronbach's α reliability coefficients for internal consistency of data were calculated taking into consideration the variables of SES, dietary consumption, household food insecurity and serum level of micronutrients. The Cronbach's α in data were $0{\cdot}8179$, $0{\cdot}8159$ and $0{\cdot}8020$, respectively, in PSAC, NPNLW and SAC, which is suggestive of strong internal consistency[24].

Results

The national prevalence of anaemia was $33{\cdot}1$ % in PSAC; the prevalence in rural, urban and slum areas was $36{\cdot}6$ %, $22{\cdot}8$ % and $22{\cdot}0$ %, respectively (Table 1). The anaemia

prevalence in NPNLW was 26·0% (national), 27·4% (rural), 21·4% (urban) and 20·1% (slum; Table 1).

The national prevalence of Fe deficiency in PSAC was 10·7% (Table 1); it was 27·2%, 9·4% and 12·3%, respectively, in slum, rural and urban strata. Fe deficiency in NPNLW was 7·1% at national level; it was 8·7%, 7·4% and 6·7% in urban, slum and rural strata, respectively. Fe deficiency in SAC was 3·9% in those aged 6–11 years and 9·5% in those aged 12–14 years (Table 2). The national prevalence of Fe-deficiency anaemia was 7·2% and 4·8%,

respectively, in PSAC and NPNLW (Table 1). Fe-deficiency anaemia national prevalence in SAC was 1·3% and 1·8%, respectively, in 6–11 and 12–14 years age groups (Table 2). The prevalence of elevated CRP (>10 mg/l) and elevated AGP (>1 g/l) was respectively 4·6% and 28·5% in PSAC; 2·2% and 15·3% in SAC; and 1·9% and 12·8% in NPNLW. The unadjusted prevalence of Fe deficiency was 9·7%, 3·9% and 7·5% in PSAC, SAC and NPNLW, respectively (see online supplementary material, Supplemental Table 3).

Table 1 Prevalence of anaemia, iron deficiency and iron-deficiency anaemia, nationally and according to stratum of residence, in pre-school age children (PSAC; 6–59 months old) and non-pregnant non-lactating women (NPNLW; 15–49 years old), Bangladesh, 2011–2012

	PSAC			NPNLW		
	n	%	95 % CI	*n*	%	95 % CI
Anaemia†						
National	607	33·1	25·7, 40·4	1031	26·0	20·2, 31·6
Rural	207	36·6	27·5, 45·6	362	27·4	20·3, 34·6
Urban	220	22·8	12·9, 32·8	351	21·4	13·7, 29·1
Slum	180	22·0	14·1, 29·9	318	20·1	12·9, 27·3
Fe deficiency‡,§						
National	468	10·7	5·8, 15·6	882	7·1	4·2, 9·9
Rural	155	9·4	3·2, 15·7	314	6·7	3·1, 10·2
Urban	164	12·3	4·3, 20·2	298	8·7	4·1, 13·3
Slum	149	27·2	19·5, 34·9	270	7·4	3·8, 11·1
Fe-deficiency anaemia‖						
National	449	7·2	2·4, 11·9	868	4·8	2·1, 7·8
Rural	149	6·1	0·02, 12·1	312	5·0	1·6, 8·4
Urban	158	10·1	2·2, 18·0	294	4·1	1·4, 6·7
Slum	142	13·9	5·5, 22·2	262	4·1	1·3, 6·8

†Anaemia is defined as Hb <12 g/dl in NPNLW and <11 g/dl in PSAC[49].
‡Fe deficiency is defined as serum ferritin <15 ng/ml in NPNLW and <12 ng/ml in PSAC[49].
§Adjusted for elevated C-reactive protein (>10 mg/l) or elevated α_1-acid glycoprotein (>1 g/l) by mathematical correction[18,19].
‖Fe-deficiency anaemia is defined as Hb <12 g/dl plus serum ferritin <15 ng/ml in NPNLW and as Hb <11 g/dl plus serum ferritin <12 ng/ml in PSAC[49].

Table 2 Prevalence of anaemia, iron deficiency and iron-deficiency anaemia, nationally and according to stratum of residence, in school age children (SAC; 6–14 years old), Bangladesh, 2011–2012

	SAC (6–11 years old)			SAC (12–14 years old)		
	n	%	95 % CI	*n*	%	95 % CI
Anaemia†						
National	995	19·1	13·1, 25·1	326	17·1	7·4, 26·6
Rural	340	21·7	13·7, 29·5	102	18·1	5·4, 30·7
Urban	342	11·8	8·3, 15·2	110	13·2	2·3, 24·1
Slum	313	13·2	7·0, 19·4	114	18·1	10·6, 25·6
Fe deficiency‡,§						
National	960	3·9	1·7, 6·1	319	9·5	−0·6, 19·7
Rural	331	4·1	1·1, 6·9	98	10·0	−0·4, 23·8
Urban	329	3·6	1·2, 5·9	112	8·1	2·1, 14·2
Slum	300	3·4	1·2, 5·5	109	8·3	1·8, 14·8
Fe-deficiency anaemia§,‖						
National	944	1·3	0·2, 2·4	312	1·8	−0·2, 3·9
Rural	324	1·1	−0·3, 2·5	97	1·8	−0·8, 4·6
Urban	325	2·1	1·1, 4·0	108	1·7	−0·6, 4·1
Slum	295	1·3	−0·1, 2·7	107	1·8	−0·3, 3·9

†Anaemia is defined as Hb <11·5 g/dl in children aged 6–11 years and as Hb <12 g/dl in children aged 12–14 years[49].
‡Fe deficiency is defined as serum ferritin <15 ng/ml[49].
§Adjusted for elevated C-reactive protein (>10 mg/l) or elevated α_1-acid glycoprotein (>1 g/l) by mathematical correction[18,19].
‖Fe-deficiency anaemia is defined as Hb <11·5 g/dl plus serum ferritin <15 ng/ml in children aged 6–11 years and as Hb <12 g/dl plus serum ferritin <15 ng/ml in children aged 12–14 years[49].

Table 3 Serum ferritin level according to groundwater iron level among pre-school age children (PSAC; 6–59 months old), school age children (SAC; 6–14 years old) and non-pregnant non-lactating women (NPNLW; 15–49 years old), Bangladesh, 2011–2012

	Serum ferritin†,‡ (ng/ml)								
	PSAC			NPNLW			SAC		
	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	*P*
PHGWI area	38·9	1·9	<0·001	67·9	2·3	<0·001	57·1	1·8	<0·001
PLGWI area	23·1	1·9		44·7	2·2		42·1	1·6	

PHGWI, predominantly high groundwater Fe; PLGWI, predominantly low groundwater Fe.
†Ferritin is adjusted for infection.
‡Log-transformed.
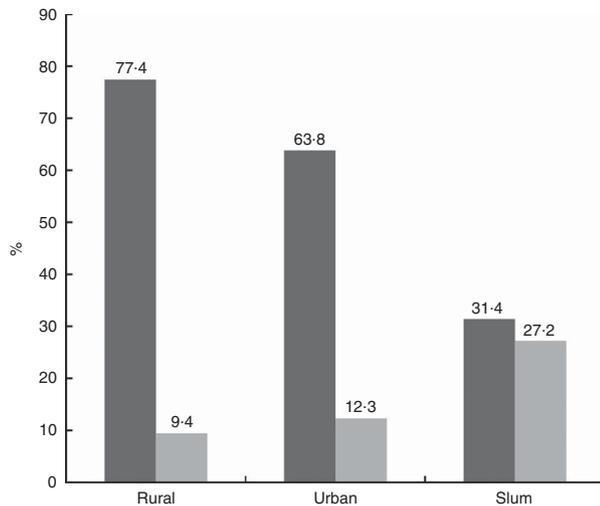
Fig. 2 Prevalence of drinking tube well water (i.e. groundwater; ▪) and of iron deficiency (▫) by area of residence among pre-school age children (PSAC; 6–59 months old) in Bangladesh, 2011–2012. An inverse trend of usage of tube well water for drinking is seen in PSAC from rural, urban and slum areas. A progressive rise in drinking of tube well water with an associated decreasing prevalence of Fe deficiency is seen from slum through urban to rural areas

Between 2·8 and 5·8 % of PSAC met the RDA for Fe[25]. The intake of animal-origin Fe accounted for 7–14 % of the RDA. A similar profile of consumption was noted in SAC and NPNLW (see online supplementary material, Supplemental Table 4).

Mean serum ferritin levels were significantly higher (*P* < 0·001) in all populations in the PHGWI areas than in the PLGWI areas (Table 3). In PSAC, prevalence of Fe deficiency and usage of tube well water (groundwater) for drinking showed an inverse relationship (Fig. 2).

Table 4 shows that household expenditure of NPNLW increased gradually as households became progressively more food secure. Similarly, there was an upward gradient in household expenditure as household wealth index increased. These observations were logically complemented by women's dietary intake of Fe, which increased as household food security and SES improved. However, serum ferritin level followed an inverse trend; generally it appeared higher in the bottom quintiles of SES

and in food-insecure households. Despite having comparable household expenditures and intakes of Fe, women from PHGWI areas had significantly higher serum ferritin than women residing in the PLGWI areas (*P* < 0·05; Table 4).

While in the older population (females aged 15–49 years), 7·9 % had risk of Fe overload, this was 1·5 % in the younger group (females aged 5–14 years). Prevalence of Fe overload risk in older women was 12·5 % and 5·3 % in PHGWI and PLGWI areas, respectively. Risk of Fe overload was more likely to occur in PHGWI areas (*P* < 0·05 in all populations), despite the fact that dietary provision of Fe was lower in those areas than in PLGWI areas. On the other extreme, prevalence of Fe deficiency was 4·7 % and 7·1 % in younger (5–14 years) and older females (15–49 years), respectively (Table 5).

The multivariate regression analyses revealed that PHGWI area was independently associated with higher serum ferritin in all population groups: standardized $\beta = 0.19$, $P = 0.03$ (NPNLW); $\beta = 0.22$, $P = 0.01$ (SAC); and $\beta = 0.20$, $P = 0.03$ (PSAC; Table 6). In PSAC, age was associated with higher levels of ferritin ($\beta = 0.30$, $P < 0.001$; Table 6) and Hb ($\beta = 0.13$, $P = 0.009$; Table 7). Age was associated with higher Hb levels in SAC ($\beta = 0.20$, $P < 0.001$). Parity (number of births) determined lower levels of ferritin in NPNLW ($\beta = -0.18$, $P = 0.006$); however, it was not associated with Hb (Tables 6 and 7). In PSAC, no measles infection within the previous 6 months was positively associated with Hb compared with measles infection (Table 7). Serum folate was associated with higher levels of ferritin in NPNLW ($\beta = 0.15$, $P = 0.02$; Table 6). Household food insecurity determined higher levels of ferritin in NPNLW ($\beta = 0.60$, $P = 0.005$ and $\beta = 0.58$, $P = 0.001$ for severe and moderate food insecurity, respectively). Dietary intake of Fe was not associated with serum ferritin ($\beta = -0.001$, $P = 0.97$) in NPNLW (Table 6). Being a female child was nearly negatively associated with serum ferritin in PSAC ($\beta = -0.13$, $P = 0.06$), while being a female child was associated with lower levels of Hb in SAC ($\beta = -0.10$, $P < 0.001$; Tables 6 and 7). Serum ferritin and retinol were independently associated with higher levels of Hb ($P < 0.05$) for all populations. Serum folate was associated with higher

Table 4 Serum ferritin level, household expenditure and dietary iron intake according to household food insecurity, socio-economic status and groundwater iron level in non-pregnant non-lactating women (15–49 years old), Bangladesh, 2011–2012

	Serum ferritin† (ng/ml)	Household expenditure‡ ($US/month)	7 d dietary iron intake (mg/d)
Household food insecurity§			
Food secure (FS)	45·1*	121·9*	54·1*
Mild food insecurity (MFI)	55·7	97·8	49·5
Moderate food insecurity (ModFI)	61·5	92·1	47·0
Severe food insecurity (SFI)	52·4	85·1	47·4
Wealth index			
Richest	49·4	192·5*	62·2*
Richer	49·9	129·7	51·8
Middle	41·6	114·2	53·5
Poorer	56·8*	95·3	48·1
Poorest	54·6*	71·2	45·7
Groundwater Fe			
PHGWI area	67·9*	98·8	49·2
PLGWI area	44·7	110·3	53·5

PHGWI, predominantly high groundwater Fe; PLGWI, predominantly low groundwater Fe.
*Significant differences, all $P < 0.05$, as follows. For household food insecurity: FS v. ModFI (serum ferritin); FS v. all the rest (household expenditure); FS v. ModFI, FS v. SFI (dietary Fe intake). For wealth index: poorer v. middle, poorest v. middle (serum ferritin); richest v. all the rest (household expenditure); richest v. poorest, richest v. poorer (dietary Fe intake). For groundwater Fe: PHGWI v. PLGWI (serum ferritin).
†Geometric mean of ferritin.
‡Geometric mean of household expenditure.
§Assessed using the Household Food Insecurity Access Scale (HFIAS)[11].

Table 5 Risk of iron overload, dietary iron intake and iron deficiency according to groundwater iron level among females (5–49 years old), Bangladesh, 2011–2012

Population	Proportion of population with risk of Fe overload† (%)			Dietary Fe intake (mg/d)		Proportion of population with Fe deficiency (%)		
	All	PHGWI area	PLGWI area	PHGWI area	PLGWI area	All	PHGWI area	PLGWI area
Females 5–14 years	1·5	4·1*	0·0	5·3*	6·5	4·7	1·0*	3·3
Females 15–49 years	7·9	12·5*	5·3	7·0*	7·7	7·1	7·6*	8·7

PHGWI, predominantly high groundwater Fe; PLGWI, predominantly low groundwater Fe.
*Significance of comparisons, all PHGWI area v. PLGWI area, as follows: For females 5–14 years: $P = 0.007$ (risk of Fe overload), $P < 0.001$ (dietary Fe intake), $P = 0.44$ (Fe deficiency). For females 15–49 years: $P = 0.01$ (risk of Fe overload), $P = 0.1$ (dietary Fe intake), $P = 0.15$ (Fe deficiency).
†Serum ferritin >150 ng/ml is defined as Fe overload[35]. Cut-off of Fe overload for children younger than 5 years is not established; therefore this group is not present in the table.

levels of Hb in NPNLW ($\beta = 0.09$, $P = 0.03$; Table 7). Intake of animal-origin Fe determined higher levels of Hb in NPNLW ($\beta = 0.12$, $P = 0.01$; Table 7). Compared with those living in rural areas, SAC living in urban areas had lower levels of ferritin ($\beta = -0.19$, $P = 0.01$); also SAC and PSAC living in urban areas had higher levels of Hb ($\beta = 0.08$, $P = 0.01$ and $\beta = 0.22$, $P < 0.001$, respectively) than their rural counterparts. Household food insecurity was associated with lower levels of Hb in NPNLW ($\beta = -0.20$, $P < 0.001$) and PSAC ($\beta = -0.21$, $P < 0.001$). PHGWI area was associated with higher Hb levels in PSAC ($\beta = 0.14$, $P = 0.01$; Table 7).

Discussion

The prevalence of Fe deficiency in Bangladesh was much less in contrast to the widely held speculation. The present findings reaffirmed the low prevalence of Fe deficiency observed in other contemporary studies in Bangladesh[3–6,26]; however, the survey also revealed low Fe deficiency at the national scale. In our study the national mean of serum ferritin was 51 ng/ml in NPNLW (data not shown), which is in agreement with the very high ferritin level (77 ng/ml) observed among rural women in a contemporary study conducted by Merrill et al.[3]. That study reported a strong, positive, dose–response association between natural Fe content in groundwater, intake of Fe from such sources and Fe status of women, concluding that the low prevalence of Fe deficiency was linked with very high concentration of Fe in the groundwater consumed. The British Geological Survey 2001 on the mineral content in groundwater across Bangladesh showed that Fe concentration is high in groundwater in most parts of Bangladesh[8]. That survey and other reports have shown that the aquifer

Table 6 Multivariate regression analyses†,‡ assessing determinants of serum ferritin in non-pregnant non-lactating women (NPNLW; 15–49 years old), school age children (SAC; 6–14 years old) and pre-school age children (PSAC; 6–59 months old), Bangladesh, 2011–2012

	NPNLW			SAC			PSAC		
	t	*P*	Standardized *β*	*t*	*P*	Standardized *β*	*t*	*P*	Standardized *β*
Covariates									
Hb	0.08	0.93	0.005	−0.06	0.95	−0.004	2.05	0.04	0.15
Household expenditure§	−1.06	0.29	−0.09	1.63	0.10	0.13	–	–	–
Woman's institutional education (ref.: no institutional education)	0.42	0.67	0.03	–	–	–	–	–	–
Agricultural profession (ref.: non-agricultural)	–	–	–	−2.18	0.03	−0.19	1.13	0.25	0.08
Age	–	–	–	–	–	–	4.42	<0.001	0.30
Female sex (ref.: male)	–	–	–	1.47	0.14	0.10	−1.9	0.06	−0.13
No diarrhoea (ref.: diarrhoea)	–	–	–	0.53	0.60	0.04	–	–	–
Wealth index (ref.: poorest)									
Poorer	2.21	0.03	0.18	–	–	–	0.30	0.76	0.03
Middle	0.75	0.45	0.06	–	–	–	−1.03	0.30	−0.09
Richer	−0.69	0.49	−0.05	–	–	–	−0.77	0.44	−0.06
Richest	2.27	0.02	0.29	–	–	–	−1.87	0.06	−0.17
Groundwater Fe (ref.: PLGWI area)									
PHGWI area	2.10	0.03	0.19	2.56	0.01	0.22	2.12	0.03	0.20
7 d total Fe intake	−0.03	0.97	−0.001	–	–	–	–	–	–
No knowledge of health benefits of eating Fe-rich foods (ref.: knowledgeable)	–	–	–	−0.60	0.55	0.04	–	–	–
Knowledge about foods that are rich in Fe (ref.: no knowledge)	−1.62	0.10	−0.11	−1.78	0.08	−0.16	–	–	–
Area of residence (ref.: rural)									
Urban	0.74	0.45	0.05	−2.52	0.01	−0.19	0.61	0.54	0.04
Slum	0.62	0.53	0.04	0.03	0.97	0.02	−0.19	0.85	−0.01
Serum retinol	−0.07	0.94	−0.004	1.48	0.14	0.10	–	–	–
Parity	−2.76	0.006	−0.18	–	–	–	–	–	–
Serum Zn	−0.35	0.72	−0.03	–	–	–	–	–	–
Household food insecurity (ref.: food secure)									
Mild food insecurity	2.26	0.02	0.20	−0.21	0.83	−0.01	1.71	0.09	0.16
Moderate food insecurity	3.38	0.001	0.58	2.06	0.04	0.20	1.36	0.17	0.27
Severe food insecurity	2.85	0.005	0.60	−0.72	0.47	−0.08	1.61	0.10	0.28
Serum vitamin B_{12}‖	1.83	0.07	0.12	–	–	–	–	–	–
Serum folate‖	2.3	0.02	0.15	–	–	–	–	–	–
Possession of refrigerator (ref.: non-possession)	2.91	0.004	0.27	–	–	–	–	–	–
Non-possession of electricity (ref.: possession)	–	–	–	2.12	0.03	0.19	–	–	–
Interaction									
(Groundwater Fe × household food insecurity	−2.33	0.02	−0.52				−1.54	0.12	−0.35

Ref., reference category; PLGWI, predominantly low groundwater Fe; PHGWI, predominantly high groundwater Fe.
†The empty fields in the table imply either not applicable or not entered in the multivariate model as the univariate or bivariate analyses were non-significant ($P > 0.05$).
‡The covariates, which were assumed to have interaction, were entered in regression as an interaction term along with the interacting predictors. Interactions of (groundwater Fe × household food insecurity) in NPNLW ($P = 0.009$) and PSAC ($P = 0.05$) were entered in the final multivariate regression; however, in the final multivariate model significant interaction was reported for the NPNLW ($P = 0.02$) while in PSAC the interaction was non-significant ($P = 0.12$). In SAC, (groundwater Fe × household food insecurity) was non-significant in the initial regression and not included in the final model. In SAC, the term (groundwater Fe × stratum) had a significant interaction ($P = 0.01$) in the initial regression; however it was not included in the final model as its inclusion would result in significant multicollinearity.
§Log-transformed for SAC.
‖Log-transformed for NPNLW.

Table 7 Multivariate regression analyses†,‡ assessing determinants of Hb in non-pregnant non-lactating women (NPNLW; 15–49 years old), school age children (SAC; 6–14 years old) and pre-school age children (PSAC; 6–59 months old), Bangladesh, 2011–2012

	NPNLW			SAC			PSAC		
	t	P	Standardized β	t	P	Standardized β	t	P	Standardized β
Covariates									
Household expenditure§,∥	−0·05	0·95	−0·003	2·17	0·03	0·07	–	–	–
Serum ferritin§,∥,¶	3·1	0·002	0·13	3·76	<0·001	0·11	3·22	0·001	0·21
Age	–	–	–	6·97	<0·001	0·20	2·63	0·009	0·13
Female (ref.: male)	–	–	–	−3·68	<0·001	−0·10	–	–	–
No fever (ref.: fever)	–	–	–	−1·94	0·06	−0·05	−0·63	0·52	−0·03
No measles (ref.: measles)	–	–	–	–	–	–	3·89	<0·001	0·20
Mother's education (ref.: no education)	–	–	–	–	–	–	0·03	0·97	0·001
7 d animal-source Fe intake§	2·46	0·01	0·12	–	–	–	–	–	–
7 d total Fe intake§	−0·46	0·64	−0·02	–	–	–	–	–	–
Parity	−1·42	0·15	−0·06	–	–	–	–	–	–
Serum folate	2·13	0·03	0·09	–	–	–	–	–	–
Serum retinol	2·93	0·004	0·13	5·14	<0·001	0·14	2·66	0·008	0·13
PHGWI area (ref.: PLGWI area)	–	–	–	–	–	–	2·57	0·01	0·14
Agricultural profession (ref.: non-agricultural)	–	–	–	−3·09	0·002	−0·09	4·74	<0·001	0·25
Area of residence (ref.: rural)									
Urban	1·52	0·13	0·06	2·58	0·01	0·08	4·03	<0·001	0·22
Slum	1·34	0·18	0·05	0·14	0·89	0·004	1·39	0·16	0·06
Wealth index (ref.: poorest)									
Poorer	−1·04	0·30	−0·05	−3·16	0·002	−0·11	0·67	0·50	0·04
Middle	−1·67	0·10	−0·09	−2·29	0·02	−0·08	1·88	0·06	0·12
Richer	−1·59	0·11	−0·09	−1·44	0·15	−0·05	−0·33	0·74	−0·02
Richest	−0·35	0·72	−0·02	−1·20	0·23	−0·05	0·01	0·99	0·001
Serum Zn	1·80	0·07	0·08	–	–	–	1·18	0·23	0·07
No mobile phone (ref.: mobile phone)	–	–	–	−1·28	0·20	−0·04	–	–	–
No refrigerator (ref.: refrigerator)	–	–	–	−1·99	0·04	−0·08	–	–	–
Household food insecurity (ref.: food secure)									
Mild food insecurity	−4·34	<0·001	−0·20	–	–	–	−0·43	0·66	−0·02
Moderate food insecurity	−1·14	0·25	−0·05	–	–	–	−3·73	<0·001	−0·21
Severe food insecurity	−0·24	0·81	−0·01	–	–	–	−0·37	0·73	−0·02
Interaction									
(Groundwater Fe × stratum)	−0·38	0·7	−0·02						

Ref., reference category; PHGWI, predominantly high groundwater Fe; PLGWI, predominantly low groundwater Fe.
†The empty fields in the table imply either not applicable or not entered in the multivariate model as the univariate or bivariate analyses were non-significant ($P > 0.05$).
‡The covariates, which are assumed to have interaction, were entered in the initial regression as an interaction term along with the interacting predictors. However, the regression coefficients for the interaction terms were non-significant ($P > 0.05$); therefore, they were not entered in the final multivariate model, except the interaction term of (groundwater Fe × stratum) in NPNLW ($P = 0.005$). However, it had no significant interaction in the final model ($P = 0.7$).
§Log-transformed for NPNLW.
∥Log-transformed for SAC.
¶Log-transformed for PSAC.

environment in Bangladesh is reducing[26,27], indicating that dissolved Fe is predominantly ferrous (Fe^{2+}), a form that is readily absorbed through the gut[28]. This was supported by an experimental study which showed that natural water with electrolytically reduced Fe (ferrous) is readily absorbed[29]. We used the data on Fe load in groundwater from the British Geological Survey 2001 and compared mean ferritin levels in our data between the PHGWI area (groundwater Fe ≥ 2·8 mg/l) and PLGWI area (groundwater Fe < 2·8 mg/l). Mean serum ferritin was statistically significantly higher in all population groups in the PHGWI area (Table 3).

Discussion on key findings of multivariate regression

Serum ferritin
Discussion in relation to serum ferritin is as follows.

1. The PHGWI area was associated with higher serum ferritin levels. This finding was observed despite the fact that SES (e.g. household expenditure) and intake of dietary Fe (total and from animal sources) were significantly lower in children living in PHGWI areas than in children living in PLGWI areas (see online supplementary material, Supplemental Table 5), suggesting that the higher Fe level in water from the PHGWI areas might have conferred a higher level of ferritin.
2. Residence in the urban stratum was associated with lower ferritin levels in SAC, compared with the rural stratum. Although household expenditure was significantly higher in urban children, lower usage of water from tube wells for drinking might have accounted for the lower ferritin in urban children compared with their rural counterparts (Supplemental Table 5).
3. Household food insecurity determined higher levels of serum ferritin in NPNLW. This result can be explained by higher prevalence of household food insecurity in the PHGWI than in the PLGWI areas. Women of food-insecure households had higher levels of ferritin despite lower household expenditure and lower dietary intake of Fe (Supplemental Table 5).
4. Non-possession of electricity in the household was associated with higher ferritin levels in SAC. A higher proportion of the population who did not have electricity in their household was residing in the PHGWI areas. SAC from households without electricity had higher serum ferritin despite having significantly lower household expenditure and dietary intake of Fe (Supplemental Table 5).

The multivariate regression results and underlying analyses into the findings (Supplemental Table 5) therefore clearly make a case for high level of Fe in groundwater as it explains the interesting observations, such as the association of higher level of household food insecurity with higher level of Fe nutrition.

We explored the intake of dietary Fe. Although consumption of animal-source foods such as meat, fish and eggs has increased in Bangladesh[30], the dietary intake of Fe is still well below the requirement. Only a small portion of the population met the RDA[25] (see online supplementary material, Supplemental Table 4). This was consistent with the findings of the multivariate regression analyses which did not find any impact of dietary Fe on serum level of ferritin (Table 6). A similar observation was noted in a contemporary study in the country[3].

The apparent higher proportion of women at risk of Fe overload (Table 5) is perhaps explained by the assumption that women are likely to consume a higher amount of water than children. This assumption is supported by evidence that women in rural Bangladesh drink nearly twice as much water as children: 2·7 v. 1·4 l/d[31]. Bangladesh has no Fe supplementation programme for women who are not pregnant and not lactating. Even in pregnant women who are targeted under the government programme, the status of Fe supplementation is bleak. Only 15 % of rural pregnant women take at least 100 tablets and 46 % take none. Access to Fe + folic acid supplementation is scarce, as only 27 % of women make three or more antenatal visits[32]. Hence, supplemental Fe intake among our study group (i.e. NPNLW) understandably would be negligible, so much so to have made a positive impact on ferritin status. In the absence of significant supplementation, higher intake of 'Fe-rich' water by NPNLW might plausibly be associated with higher ferritin status, leading to a higher proportion of them being at risk of having Fe overload.

The groundwater Fe concentration in a north-western district in Bangladesh was 16·7 mg/l, a level over fifty times the WHO-defined aesthetic limit of 0·3 mg/l[26]. The daily intake of Fe from groundwater was 42 mg/d in women[3], which is near the limit of 45 mg/d as the tolerable upper intake level from foods, water and supplements combined[33]. The research on groundwater quality in Bangladesh suggests that the ratio of ferrous (bioavailable form of Fe) to ferric (Fe^{3+}) is large[28]. Additionally, a study that sampled groundwater in Bangladesh reported that nearly all (99 %) of the total Fe in water is dissolved and thus readily absorbable[27]. It is important to note that most of the drinking-water (60 %) in Bangladesh is consumed within 5 min of pumping from a tube well, before light, air and temperature can oxidize the ferrous to the ferric form[34]. Therefore, in view of the predominant presence of the ferrous form of Fe and the prime practice of drinking freshly pumped water, intake of bioavailable Fe from groundwater appears to be high in the Bangladeshi population.

The analyses explicitly indicate that the widespread presence of high Fe level in groundwater might have contributed to higher ferritin level in the Bangladeshi population, irrespective of socio-economic condition, area

of residence, household food insecurity and status of dietary consumption. In a state of inadequacy of dietary Fe, limited food fortification programmes (with a limited coverage of micronutrient powder supplementation in young children; ~1 % in the present survey data (results not shown)) and an inadequate coverage of Fe + folic acid supplementation in women, high level of Fe in groundwater appeared to have a reasonable association with higher Fe status. We used serum ferritin < 15 ng/ml to define Fe deficiency in NPNLW and SAC because this cut-off is the best predictor of Fe deficiency[35]. In the event of considering the cut-off as serum ferritin <12 ng/ml used in other studies[3], the prevalence of Fe deficiency would have been even lower.

The difference in prevalence of anaemia in the present survey compared with other contemporary national surveys[1] is perhaps due to the difference in assessment methods. The national micronutrients survey used venous blood samples analysed with Hemocue, whereas capillary blood analysis by Hemocue is commonly used to assess anaemia in Bangladesh and other regions. We plotted Hb values against serum ferritin; ferritin was positively correlated with Hb in all study groups (see online supplementary material, Supplemental Fig. 1). Another study noted a similar positive correlation between Hb and ferritin[36].

Hb

Discussion in relation to Hb is as follows.

1. Serum ferritin was associated with higher levels of Hb in PSAC. This finding is complemented by the significant correlation observed between Hb and ferritin (see online supplementary material, Supplemental Fig. 1).
2. Serum retinol was associated with higher levels of Hb. This finding reiterates the role of retinol in Hb synthesis (Supplemental Table 6). The prevalence of anaemia was 19 %, 33 % and 17 % higher, respectively, in NPNLW, PSAC and SAC with retinol deficiency $v.$ their non-deficient counterparts (data not shown). The positive association of retinol and Hb has been documented in several other studies[37,38]. Potential mechanisms by which vitamin A may contribute to reduce anaemia could be mobilization of Fe stores from the liver, increased erythropoiesis and the lessening of infection.
3. Serum Zn and Hb had a weakly positive association in NPNLW. Anaemia prevalence was 11 % higher in NPNLW with Zn deficiency compared with no deficiency (data not shown). The underlying mechanism is unclear; however, it is perhaps explained by common dietary sources of Zn and Fe and the role of Zn in erythropoiesis[39,40].
4. Serum folate was positively associated with Hb in NPNLW. This observation corresponded to higher serum folate in non-anaemic than in anaemic women (Supplemental Table 6). Folate deficiency has been found to be associated with anaemia in women in Bangladesh[4].
5. Intake of animal-source Fe was associated with higher levels of Hb in NPNLW. This finding is complemented by the observation that intake of animal-origin Fe was higher in non-anaemic than in anaemic women (Supplemental Table 7). Similarly, another study in Bangladesh has reported that bioavailable Fe is a significant predictor of Hb status in women[41]. These findings have policy significance in relation to the national anaemia control strategy.
6. Being a female child and living in an urban area were associated with lesser and higher status of Hb, respectively. The explanation behind this is perhaps the significantly lower intake of micronutrients potentiating the synthesis of Hb (e.g. Fe and vitamin A) by the female and higher intake of the same by the urban children (Supplemental Table 7). A similar finding has been noted in the USA, where females were found to consume less micronutrients than males[42].
7. Moderate and severe grades of household food insecurity did not predict lower Hb levels in NPNLW. This finding is despite the fact that the intake of animal-source Fe by women from food-insecure households was significantly less than that of women who were food secure. The finding is plausibly explained by the observation of higher serum ferritin in women from food-insecure households and the higher proportion of food-insecure households residing in PHGWI areas (Supplemental Table 7). There was some interaction of groundwater Fe and household food insecurity in determining Hb level in women; however, it was not significant ($t = 1.32$, $P = 0.18$; data not shown).
8. PHGWI area predicted higher levels of Hb in PSAC. The finding is supported by lower anaemia prevalence in the PHGWI areas compared with the PLGWI areas (17 % $v.$ 34 %, $P < 0.05$; data not shown). A similar observation has been noted in another study in Bangladesh, which reported that intake of groundwater with elevated Fe content was associated with a 44 % reduction in the odds of anaemia in women[43]. This is important for the strategy in relation to anaemia and Fe nutrition policy in Bangladesh.

Alongside Fe the other issues in relation to Hb

The finding of a modest prevalence of Fe deficiency complements the observation of a moderate magnitude of anaemia in Bangladesh. Fe deficiency is widely believed to be the commonest cause of anaemia. However, Fe deficiency as cause of anaemia appeared small in the present study, perhaps resulting in a relatively lower prevalence of anaemia. Despite this, the prevalence of anaemia was more than three times that of Fe deficiency in children and women, suggesting other underlying factors. Our data revealed that, in addition to dietary Fe, vitamin A,

folate and Zn are important determinants of Hb in Bangladesh. Similarly an earlier study[6] reported that, alongside Fe, vitamin A was critical for determining higher levels of Hb. The other condition that might be important to cause anaemia is congenital Hb disorders. In Bangladesh no nationally representative data are available reporting the magnitude of haemoglobinopathies; however, a recent study suggested that it can be quite high, e.g. 28 % among women[43]. The link between anaemia and congenital Hb disorders has been reported in Bangladesh and in the neighbouring countries of Vietnam and Cambodia[43]. Finally, our study revealed that high levels of Fe in groundwater were associated with higher Hb status in young children.

Fe and multiple micronutrient interventions for controlling anaemia and Fe deficiency in the perspective of high Fe level in groundwater

In light of a modest prevalence of Fe deficiency and its association with high Fe level in groundwater in the present and other studies[3,8,26,35,43], it is pertinent to revisit the scope of Fe supplementation programmes, including those with micronutrient powders, for any future micronutrient strategy of Bangladesh. By using the WHO cut-off, up to 7·9 % of women had serum ferritin higher than the level defining Fe overload (>150 ng/ml). The WHO has not defined a cut-off limit for young children for Fe overload; however, our data suggest that from one-half up to two-thirds of the children had serum ferritin above the normal reference limit[44] (see online supplementary material, Supplemental Table 8). This illustrates that a considerable proportion of children are at risk of getting excess Fe in the body. Chronic exposure to excess Fe can lead to health hazards[45] such as gastrointestinal distress[34], untoward shift in gut microbiota[46] and suppressed Zn absorption[47]. In this connection, the WHO guidelines on supplementation with micronutrient powders states that the intervention should be preceded by an evaluation of the nutritional status (i.e. micronutrients status) to ensure that daily micronutrient needs are met and not exceeded[48]. This is pertinent in Bangladesh against the backdrop of high levels of Fe in groundwater and presumably a high intake of Fe from drinking such water.

High Fe level in groundwater, with its positive association with population Fe status, can have potential implication in anaemia prevention programmes. In the PHGWI areas, Fe or multiple micronutrient supplementations targeting reduction of anaemia might have a less efficient impact because the population might already be Fe sufficient at the outset. Besides, supplementation would further add to an already excess load of Fe with potential health hazards.

High level of Fe in groundwater may not be a situation unique to Bangladesh; perhaps in other countries the same situation might prevail. Similar to Bangladesh, there is apparently a high level of Fe in groundwater in South-East Asian countries with a low prevalence of Fe deficiency (see online supplementary material, Supplemental Table 9). This observation promises to open up a new research agenda and policy discussion globally to fine tune anaemia control strategies around the world.

It is essential that anaemia and multiple micronutrient deficiency control interventions in Bangladesh and similar settings are guided by evaluation of: (i) the concentration of Fe in groundwater and attribution of that to total Fe intake; (ii) the status of Fe and other relevant micronutrients (e.g. vitamin A, Zn, folate, vitamin B_{12}); (iii) dietary micronutrient intake sorted by animal *v.* plant sources; and (iv) congenital Hb disorders. The evaluation would inform specific interventions customized by geographical area, modification of supplementation preparations if required, or provide suggestion for new interventions. This is likely to make anaemia and micronutrient interventions of the country fully sensitive to needs, efficient, impactful and cost-efficient, and would avoid potential health issues.

Limitation of the study

In the present study no direct estimation of Fe concentration in groundwater was done; hence the association of groundwater Fe with population Fe status needs cautious interpretation. We referred to a nationwide smoothed map prepared by the British Geological Survey of 2001[8] for estimation of groundwater Fe load in Bangladesh. The smoothed maps were derived by kriging – a well-documented geo-statistical method used to estimate values, in this case groundwater Fe concentration, in non-sampled areas using data from sampled points – that is quite successful in interpolating information on the spatial structure of mineral concentration[26].

Conclusion

In conclusion, Fe deficiency appeared not to be a major public nutrition problem in Bangladesh; high level of Fe in groundwater was associated with higher Fe status in the population. The research highlighted the environmental issue of groundwater Fe and informs policy makers and programme managers about the importance of considering it in planning, designing and evaluating micronutrient supplementation and anaemia control programmes, to optimize the interventions at the appropriate level, in Bangladesh and globally.

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Supplementary material

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