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Plasma zinc, vitamin B_{12} and α-tocopherol are positively and plasma γ-tocopherol is negatively associated with Hb concentration in early pregnancy in north-west Bangladesh

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Abstract

Objective: The objective of the current analysis was to explore the association of multiple micronutrients with Hb concentration among pregnant women in a South Asian setting, a topic that has not been adequately explored.
Design: Sociodemographic, anthropometric and micronutrient status (plasma ferritin, transferrin receptor, retinol, α- and γ-tocopherol, folate, vitamin B_{12}, Zn) and Hb concentration were assessed at early pregnancy.
Setting: The biochemical sub-study was nested within a double-blind, placebo-controlled, community-based vitamin A and β-carotene supplementation trial in rural north-western Bangladesh (JiVitA). All assessments were conducted before trial supplementation was initiated.
Subjects: A systematic sample of 285 women was selected from those enrolled in the biochemical sub-study.
Results: Seventeen per cent of women were mildly anaemic; moderate and severe anaemia was uncommon (2·1 %). α-Tocopherol, vitamin B_{12} and Zn deficiencies were common (43·5 %, 19·7 % and 14·7 %, respectively); however, vitamin A, folate and Fe deficiencies were comparatively rare (7·4 %, 2·8 % and <1 %, respectively). Plasma Zn, vitamin B_{12} and α-tocopherol were positively associated and plasma γ-tocopherol was negatively associated with Hb ($P < 0·05$) after adjustment for gestational age, inflammation status, season and nutritional status measured by mid-upper arm circumference.
Conclusions: Among pregnant women in rural Bangladesh with minimal Fe deficiency, plasma Zn, vitamin B_{12}, and α- and γ-tocopherol concentrations were associated with Hb concentration. Appreciating the influence on Hb of micronutrients in addition to those with known associations with anaemia, such as Fe, folate, and vitamin A, is important when addressing anaemia in similar settings.

Keywords
Hb
Tocopherol
Zinc
Pregnancy

The global prevalence of anaemia among pregnant women, a demographic that experiences a disproportionately elevated risk, is estimated to be 42 % or more[1]. In Bangladesh, the most recent national survey from 1998 to 1999 showed that 49 % of pregnant women were anaemic, similar to the rate found in other studies and surveys across the country[2–4].

Anaemia is known to increase the risk of maternal mortality, preterm delivery and low-birth-weight infants[5,6]. Most often efforts to control anaemia, especially among women of reproductive age, are aimed at reducing the risk of Fe deficiency, the world's most common single micronutrient deficiency and the leading cause of anaemia, associated with half of all cases[1]. However, despite diets with apparently poor Fe availability[7], recent data from our study site in north-western Bangladesh suggest that Fe deficiency is actually uncommon, with women likely consuming substantial bioavailable Fe through well water[8].

Among other factors that can lead to anaemia, including parasitic infections[9] and genetic disorders affecting erythrocyte production and survival[10], nutritional deficiencies in addition to that of Fe can negatively impact erythropoiesis. Vitamin A (retinol) deficiency impairs

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erythrocyte differentiation and proliferation[11]. A deficiency in α-tocopherol (vitamin E), one of the most potent fat-soluble antioxidants important for erythrocyte membrane maintenance, can lead to haemolysis[12]. Vitamin B_{12} and folate deficiencies will impair DNA synthesis and cause ineffective erythropoiesis[13]. Inadequate Zn status may contribute to the burden of anaemia by limiting erythropoiesis or by decreasing erythrocyte resistance to oxidative stress[14].

In many developing countries, pregnant women experience multiple micronutrient deficiencies which may contribute to anaemia[15]. Despite the wealth of data on anaemia prevalence in Bangladesh, there is a dearth of information on the extent to which micronutrient deficiencies beyond poor Fe status are associated with circulating Hb as women begin the nutritionally and physiologically demanding period of pregnancy. To elucidate the aetiology of low Hb concentration specifically related to nutritional deficiencies beyond Fe among women in the physiologically demanding period of pregnancy, the objective of the current analysis was to explore the association of vitamin A, vitamin B_{12}, α- and γ-tocopherol, folate and Zn status with Hb concentration among a population of pregnant women in rural Bangladesh.

Experimental methods

Study design

Data were collected in the context of a large, double-blind, cluster-randomized, placebo-controlled vitamin A and β-carotene supplementation trial (JiVitA-1 trial) implemented in rural north-west Bangladesh from 2001 to 2007. Details of the trial were published elsewhere[16,17]. In brief, once enrolled in early pregnancy, a baseline interview was conducted to collect, among other information, demographic and socio-economic status as well as reproductive history. Diet was assessed using a twenty-eight-item, 7 d FFQ. Mid-upper arm circumference (MUAC) was measured to the nearest 0·1 cm using a non-stretchable, locally manufactured insertion tape. All data were collected by locally hired, trained, female staff.

Within this setting, a biochemical sub-study that involved more intensive assessments of nutritional and health status, including home-based collection of a non-fasting venous blood sample, was initiated in 5 % of the area, purposively selected. Blood samples collected at the first assessment (at median gestational age of 10 (interquartile range 8–12) weeks), before initiation of trial supplementation, were immediately stored in a cool insulation bag for same-day transport to and processing at the local field laboratory in Gaibandha. Plasma samples were stored in liquid nitrogen immediately after separation and through transport to Johns Hopkins University, Baltimore, MD, USA, where they were stored until analysis. Hb concentration (g/l) was assessed on the spot using a B-Hemoglobin Analyzer (HemoCue Inc., Angelholm, Sweden).

Plasma ferritin, C-reactive protein (CRP), folate and vitamin B_{12} were assessed using an Immulite 1000 chemiluminescent immunoassay system (Diagnostic Products Corporation, Los Angeles, CA, USA). Zn was analysed by atomic absorption spectrometry (AAnalyst800; Perkin Elmer, Waltham, MA, USA). Transferrin receptor (TfR) was assessed using a commercial ELISA kit (Ramco Laboratories, Inc., Stafford, TX, USA). α_1-Acid glycoprotein (AGP) was measured using a radial immunodiffusion assay (Kent Laboratories, Bellingham, WA, USA). These assays were performed at the Johns Hopkins University Center for Human Nutrition on a representative subset of all the available samples, while retinol and tocopherols were assessed on all samples. Retinol, α-tocopherol and γ-tocopherol were determined simultaneously by reverse-phase HPLC at Mahidol University in Thailand using the method of Yamini *et al.*[18]. The within- and between-assay CV for plasma samples were <10 % for all analytes except α- and γ-tocopherol, for which inter-assay CV were ~20 %.

Selection of participants

Women included in the present analysis represent a systematically derived sub-sample of one in every five participants of the biochemical sub-study for whom an early pregnancy blood sample was available at the Johns Hopkins University laboratory when the laboratory analysis commenced. The sub-sample was selected by choosing every fifth sample after ordering them chronologically and using a random start.

Statistical analysis

Socio-economic status was described by quartile of living standard index which was defined for the entire study population at baseline[19]. General wasting undernutrition was defined as MUAC < 21·5 cm[17]. Diet patterns were defined by categorizing 7 d intake frequency into three or more times *v.* fewer than three times for each of seven food groups: any meat or poultry, fish, egg, dairy, dark green leafy vegetables, other vegetables and fruits. To capture possible seasonality of food intake and susceptibility to infection associated with season of blood sample collection, six standard seasons were defined using the Bangladeshi calendar: winter, spring, summer, early monsoon, late monsoon and autumn.

Geometric means and 95 % confidence intervals were used to describe plasma ferritin, TfR, γ-tocopherol, folate, vitamin B_{12} and CRP concentrations by anaemia status as they had skewed distributions. Anaemia was defined by Hb concentration as: mild, <110 g/l or <105 g/l for gestational age <12 weeks or ≥12 weeks, respectively[20]; moderate, <90 to 70 g/l; and severe, <70 g/l. Micronutrient deficiency was defined with the following cut-offs: Fe deficiency, ferritin <12 μg/l or TfR >8·5 mg/l; retinol,

$<0{\cdot}70$ μmol/l[18]; α-tocopherol, $<9{\cdot}3$ μmol/l[21]; vitamin B_{12}, <150 pmol/l[22]; folate, $<6{\cdot}7$ nmol/l[23]; and Zn, $<8{\cdot}6$ μmol/l[24]. An additional less conservative cut-off of <12 μmol/l was also used to define α-tocopherol deficiency[25]. Four stages of infection and inflammation were used following the categorization recommended by Thurnham *et al.*[26]: normal, no elevated CRP ($>5{\cdot}0$ mg/l) or AGP ($>1{\cdot}0$ g/l); incubation, elevated CRP but normal AGP; early convalescence, elevated CRP and AGP; and late convalescence, elevated AGP but normal CRP.

Rates of anaemia across baseline characteristics were tested using the χ^2 test or Fisher's exact test, as appropriate. Hb and micronutrient concentrations were compared across anaemia status and other categories using Student's *t* test or one-way ANOVA. Pearson's correlation coefficient was used to investigate the associations between plasma micronutrient concentrations and Hb concentration.

Linear regression was used to examine the strength of the associations between plasma micronutrient concentrations and baseline characteristics and Hb concentration as the dependent variable. All micronutrient concentrations were standardized to a *Z*-score to improve the interpretability of interactions. Characteristics associated with Hb concentration ($P<0{\cdot}20$) in bivariate analyses were considered as potential confounders. Two-way interactions between micronutrients were tested with Hb concentration and retained for further analysis if significant ($P<0{\cdot}05$). Two regression models are presented: (i) a base model with all seven micronutrients and an interaction between Zn and vitamin B_{12}; and (ii) a model additionally adjusted for selected baseline characteristics including gestational age (weeks), MUAC $<21{\cdot}5$ cm, inflammation status (four stages) and season. The presence of multicollinearity between the micronutrients was explored by calculating variance inflation factors which were all $<2{\cdot}0$, suggesting no multicollinearity[27]. All analyses were conducted using R[28].

The study was 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, USA. Verbal informed consent was obtained from all participants in the presence of a witness and was formally recorded.

Results

Baseline characteristics of the participants included in the present analysis did not differ from those of the remaining sub-study participants (data not shown). Of those included, nearly half were younger than 20 years of age (46·1 %) and a similar proportion was nulliparous (46·0 %; Table 1). Forty-one per cent had no formal schooling. Twenty-eight per cent were classified as undernourished by a low MUAC.

Mean Hb concentration was 119·4 (SD 14·2) g/l and 19·3 % were anaemic. The vast majority of anaemia was mild as only 1·4 % and 0·7 % were found to have moderate and severe anaemia, respectively (Table 1). Hb was lower among women with gestational age ≥12 weeks ($P<0{\cdot}001$); however, the prevalence of anaemia did not differ across the same categorization. The stage of inflammation did not have an effect on Hb ($P=0{\cdot}07$). Although Hb did not differ significantly by season, there was a trend toward higher rates of anaemia during early monsoon (27·5 %), late monsoon (22·0 %) and autumn (31·2 %) compared with winter through summer months ($P=0{\cdot}002$ from χ^2 test for trend).

Fish was the most frequently consumed animal food; over half (55·4 %) of the women ate fish regularly, i.e. three or more times in the previous 7 d. Only 22·3 % and 36·5 % of the women reported consuming leafy vegetables and fruits three or more times weekly, respectively. None of the other food groups were commonly consumed on a regular basis. However, in combination, any animal-source foods were consumed regularly by a majority of the women (87·6 %). The Hb concentration and anaemia prevalence did not differ for any food group by category of intake frequency (see Supplementary Materials, Supplementary Table 1).

Overall the most common micronutrient deficiencies were those of α-tocopherol (43·5 %), vitamin B_{12} (19·7 %) and Zn (14·7 %). When using a less conservative cut-off of 12 μmol/l, α-tocopherol deficiency was 70·2 % prevalent. Fe, retinol and folate deficiencies were uncommon (<1 %, 7·4 % and 2·8 %, respectively). Only the prevalence of Zn deficiency was significantly different by anaemic status ($P<0{\cdot}001$; Table 2).

Hb was positively correlated with retinol ($r=0{\cdot}14$, $P<0{\cdot}05$), vitamin B_{12} ($r=0{\cdot}15$, $P<0{\cdot}05$) and Zn ($r=0{\cdot}28$, $P<0{\cdot}001$), but not with ferritin ($r=0{\cdot}04$, $P>0{\cdot}05$) or folate ($r=0{\cdot}10$, $P>0{\cdot}05$; see Supplementary Materials, Supplementary Figure 1). Hb concentration was significantly higher in the highest quartile compared with the lowest quartile for retinol, α-tocopherol, vitamin B_{12} and Zn concentrations (Fig. 1).

Nearly a third of women (36·8 %) had no micronutrient deficiency while 43·9 % had one and 19·3 % had two or more concurrent micronutrient deficiencies (Table 3). Hb concentration decreased significantly in a dose–response manner from 122·6 g/l to 111·3 g/l as the number of concurrent micronutrient deficiencies increased to three or more ($P<0{\cdot}001$).

In linear regression analysis including all seven micronutrients and the interaction between Zn and vitamin B_{12}, only Zn and vitamin B_{12} were associated ($P<0{\cdot}001$) with Hb concentration (Table 4; for standardized variables: $\beta=0{\cdot}23$ (95 % CI 0·12, 0·35) and $\beta=0{\cdot}16$ (95 % CI 0·04, 0·27), respectively). After additionally adjusting for selected baseline characteristics including undernutrition (MUAC $<21{\cdot}5$ cm), gestational age (weeks)

Table 1 Characteristics, prevalence of anaemia and Hb concentration of the study participants: women in early pregnancy (*n* 285) in rural Bangladesh

	Total		Anaemia†		Hb (g/l)	
Characteristic	*n*	%	*n*	%	Mean	SD
Overall	285	100·0	55	19·3	119·4	14·2
Age (years)‡						
<20	131	46·1	21	16·0	121·0	13·2
20–29	114	40·1	26	22·8	117·9	15·2
≥30	39	13·7	8	20·5	118·4	14·4
Parity						
0	131	46·0	23	17·6	119·7	13·4
1	67	23·5	15	22·4	119·4	14·1
≥2	87	30·5	17	19·5	118·9	15·7
Education‡						
No formal schooling	116	40·8	22	19·0	119·4	14·1
Primary	60	21·1	13	21·7	118·2	13·4
Secondary or more	108	38·0	20	18·5	119·9	14·9
Living standard index (quartile)‡,§						
1	71	25·0	13	18·3	121·3	14·3
2	72	25·4	11	15·3	119·7	11·7
3	69	24·2	16	23·2	117·7	15·8
4	72	25·4	15	20·8	118·7	14·9
Mid-upper-arm circumference (cm)						
<21·5	80	28·2	20	25·0	116·9	15·5
≥21·5	205	71·9	35	17·1	120·3	13·6
Season‖						
Winter	39	13·7	4	10·3*	118·5	13·1
Spring	46	16·1	3	6·5	124·0	12·7
Summer	58	20·4	11	19·0	120·1	14·9
Early monsoon	51	17·9	14	27·5	119·1	17·1
Late monsoon	59	20·7	13	22·0	117·2	11·4
Autumn	32	11·2	10	31·3	116·7	15·3
Gestational age at blood collection (weeks)‡						
<12	231	81·3	43	18·6	121·2	14·0*
≥12	53	18·7	12	22·6	111·6	12·8
Inflammation status¶						
Normal	195	68·4	33	16·9	120·5	13·0
Incubation	5	1·8	1	20·0	121·0	19·9
Early convalescence	14	4·9	5	35·7	110·2	15·4
Late convalescence	71	25·0	16	22·5	117·8	16·3

*$P < 0·01$ for difference across categories using the χ^2 test or Fisher's exact test for anaemia prevalence and Student's *t* test or ANOVA for Hb concentration.
†Anaemia was defined as Hb <110 g/l or <105 g/l for gestational age <12 weeks or ≥12 weeks, respectively[20].
‡Missing value for age (*n* 1), education (*n* 1), living standard index (*n* 1) and gestational age (*n* 1). Percentage calculation based on those with data.
§Living standard was defined for the entire study population at baseline using an algorithm developed with principal component analysis[19].
‖Six seasons (starting from the middle of December) were defined using the Bangladeshi calendar: winter, spring, summer, early monsoon, late monsoon and autumn.
¶Inflammation status defined as: normal, no elevated C-reactive protein (CRP; >5·0 mg/l) and no elevated α_1-acid glycoprotein (AGP; >1·0 g/l); incubation, elevated CRP but normal AGP; early convalescence, elevated both CRP and AGP; late convalescence, elevated AGP but normal CRP.

and inflammation status (four stages), Zn, vitamin B_{12} and α-tocopherol were positively associated ($\beta = 0·15$ (95 % CI 0·04, 0·27), $\beta = 0·15$ (95 % CI 0·03, 0·27) and $\beta = 0·17$ (95 % CI 0·04, 0·31), respectively) and γ-tocopherol was negatively associated ($\beta = -0·14$ (95 % CI −0·27, −0·02)) with Hb concentration. The interaction term between Zn and vitamin B_{12} was also negatively associated with Hb concentration ($\beta = -0·17$ (95 % CI −0·28, −0·06)).

Discussion

In a typical rural Bangladesh setting, we found anaemia (19·3 %) amidst Fe sufficiency during early pregnancy. Most of the anaemia was mild in nature as the prevalence of moderate and severe anaemias was rare. Nearly half of the women had at least one micronutrient deficiency with the most common being those of α-tocopherol, vitamin B_{12} and Zn. In adjusted analyses, these three micronutrients were positively associated and γ-tocopherol was negatively associated with Hb concentration. It was also found that increasing numbers of concurrent deficiencies likely contributed to reduced Hb concentrations during this physiological stage.

The prevalence of Fe deficiency (<1 %) was much lower than expected among these women living in a resource-poor setting where meat consumption is rare and diets are high in Fe-absorption inhibitors[7]. We have previously shown that Fe deficiency is low among this population most likely as a result of the chronic consumption of naturally Fe-rich groundwater used for drinking and cooking, which was found to be significantly

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Table 2 Plasma micronutrient concentrations and prevalences of deficiency by anaemia status among women in early pregnancy (n 285) in rural Bangladesh

	Overall		Non-anaemic		Anaemic†	
Indicator	Mean or n	95 % CI or %	Mean or n	95 % CI or %	Mean or n	95 % CI or %
n (%)	285	100·0	230	80·7	55	19·3
Ferritin (μg/l)‡	81·1	74·8, 87·9	81·9	75·2, 89·2	77·7	62·2, 97·2
n (%) <12 μg/l	2	0·7	0	0·0	2	3·6
Transferrin receptor (mg/l)‡	3·7	3·6, 3·8	3·6	3·5, 3·8	3·8	3·5, 4·3
n (%) >8·5 mg/l	2	0·7	1	0·4	1	1·8
Retinol (μmol/l)§	1·1	1·1, 1·2	1·1	1·1, 1·2	1·1	1·0, 1·2
n (%) <0·70 μmol/l	21	7·4	19	8·3	2	3·6
α-Tocopherol (μmol/l)§	10·4	10·0, 10·8	10·4	10·0, 10·9	10·1	9·1, 11·0
n (%) <9·3 μmol/l‖	124	43·5	95	41·3	29	52·7
γ-Tocopherol (μmol/l)‡	0·7	0·7, 0·8	0·8	0·7, 0·8	0·7	0·6, 0·8
No abnormal cut-off						
Folate (nmol/l)‡	17·9	17·0, 18·8	18·0	17·0, 19·0	17·5	15·4, 20·0
n (%) <6·7 nmol/l	8	2·8	7	3·0	1	1·8
Vitamin B_{12} (pmol/l)§	206·3	196·7, 216·4	206·9	195·8, 218·6	204·0	185·8, 224·0
n (%) <150 pmol/l	56	19·7	48	20·9	8	14·6
Zinc (μmol/l)§,¶	10·8	10·6, 11·0	11·0	10·7, 11·2	10·1	9·5, 10·7
n (%) <8·6 μmol/l	42	14·7	24	10·4	18	32·7

†Anaemia was defined as Hb <110 g/l or <105 g/l for gestational age <12 weeks or ≥12 weeks, respectively[20].
‡Geometric mean (95 % CI).
§Mean (95 % CI).
‖$P<0·20$ for difference between anaemic and non-anaemic by Student's t test or the χ^2 test.
¶$P<0·01$ for difference between anaemic and non-anaemic by Student's t test or the χ^2 test.

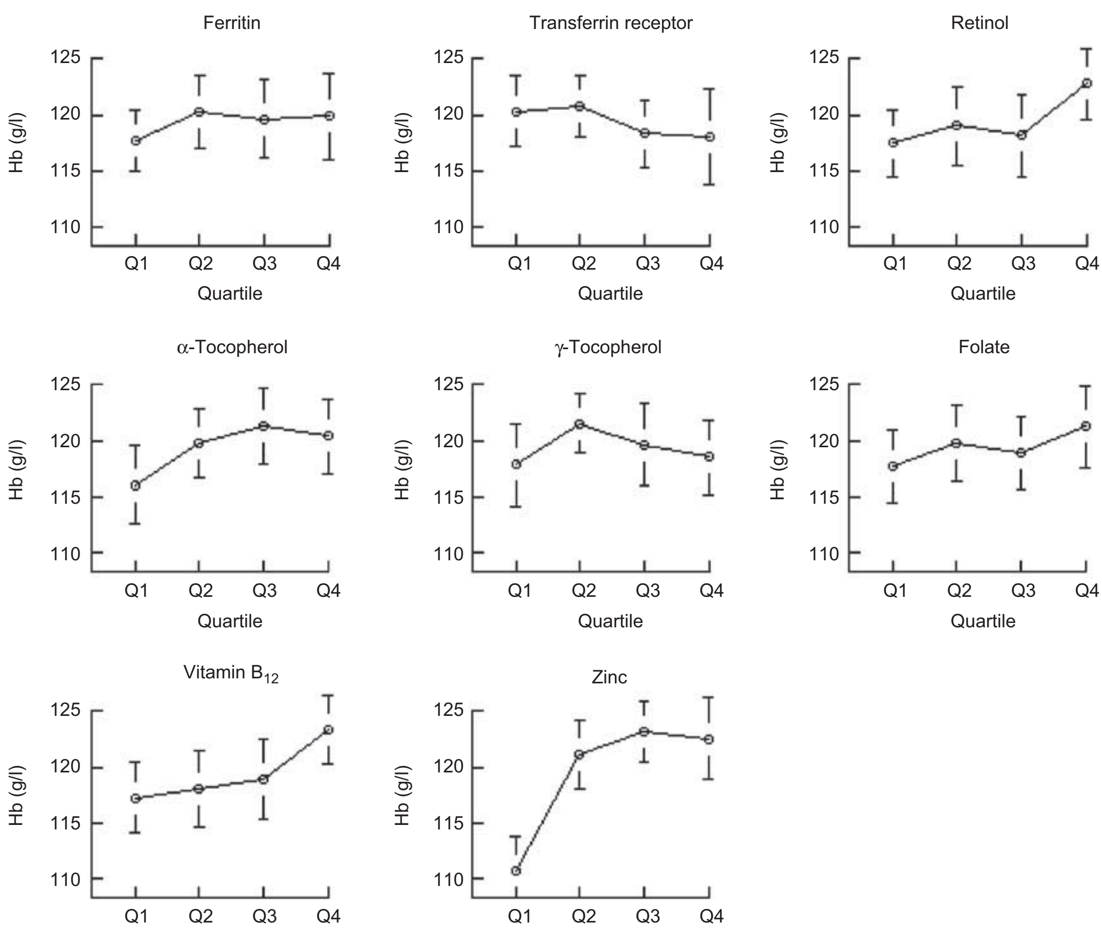

Fig. 1 Hb concentrations according to quartiles of plasma micronutrient concentrations among women in early pregnancy (n 285) in rural Bangladesh. Values are means with their 95 % confidence intervals represented by vertical bars

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Table 3 Hb concentration (g/l) by number of concurrent micronutrient deficiencies and anaemia status among women in early pregnancy (*n* 285) in rural Bangladesh

Number of micronutrient deficiencies	Overall				Non-anaemic				Anaemic†			
	n	%	Mean	95 % CI	*n*	%	Mean	95 % CI	*n*	%	Mean	95 % CI
0	105	36·8	122·6	119·9, 125·2	90	85·7	125·8	123·7, 128·0	15	14·3	103·1	98·4, 107·7
1	125	43·9	119·9	117·5, 122·3	102	81·6	124·8	122·7, 126·8	23	18·4	98·4	94·7, 102·2
2	38	13·3	112·2	107·8, 116·6	24	63·2	119·3	115·1, 123·5	14	36·8	100·1	95·4, 104·9
3 or more	17	6·0	111·3	104·7, 117·9	14	82·4	116·0	110·5, 121·5	3	17·6	89·3	78·9, 99·7
P‡			<0·001				<0·001				<0·001	

†Anaemia was defined as Hb <110 g/l or <105 g/l for gestational age <12 weeks or ≥12 weeks, respectively[20].
‡ANOVA test.

Table 4 Associations between micronutrient status and Hb concentration (g/l) by linear regression among women in early pregnancy (*n* 285) in rural Bangladesh†

Micronutrient	Model 1‡		Model 2‡	
	Standardized β	95 % CI	Standardized β	95 % CI
Ferritin (μg/l)	0·02	−0·09, 0·13	−0·01	−0·12, 0·10
Retinol (μmol/l)	0·08	−0·04, 0·21	0·01	−0·11, 0·14
α-Tocopherol (μmol/l)	0·12	−0·02, 0·26	0·17	0·04, 0·31***
γ-Tocopherol (μmol/l)	−0·12	−0·24, 0·01	−0·14	−0·27, −0·02***
Folate (nmol/l)	0·07	−0·04, 0·18	0·05	−0·06, 0·15
Vitamin B_{12} (pmol/l)	0·16	0·04, 0·27**	0·15	0·03, 0·27***
Zinc (μmol/l)	0·23	0·12, 0·35*	0·15	0·04, 0·27**
Vitamin B_{12} × Zn	−0·17	−0·29, −0·06**	−0·17	−0·28, −0·06**
R^2	0·15		0·23	

**P* < 0·05, ***P* < 0·01, ****P* < 0·001.
†Data are presented as β coefficient (95 % confidence interval) for Hb (g/l). All micronutrient concentrations were standardized to a *Z*-score.
‡Models adjusted as follows: Model 1, all seven micronutrients adjusted for an interaction term of vitamin B_{12} and Zn found to be the only one significant ($P < 0·05$) among all possible first-order micronutrient interactions; Model 2: additionally adjusted for gestational age (weeks), mid-upper arm circumference (<21·5 cm), inflammation status (four stages) and season.

associated with women's Fe status[8]. Probably due to the virtual absence of Fe deficiency, Hb concentration was not associated with Fe status among this population.

These participants experienced less common deficiencies of Fe (<1 %), Zn (14·7 %), folate (2·8 %) and vitamin B_{12} (19·7 %) compared with other parts of Bangladesh, where in contrast 8 %, 55 %, 18 % and 46 % women, respectively, were deficient[4]. Vitamin B_{12} and Zn deficiencies may be less common due to a modest but regular intake of animal-source foods. Folate deficiency was virtually absent (2·8 %) likely resulting from common consumption of folate-rich foods like pulses, a typical component of the rice-based South Asian diet. The fact that B_{12} but not folate status was associated with Hb may be due to a low rate of folate deficiency among these women.

Zn status was found to have a positive influence on Hb concentration in crude and adjusted linear regression analyses, similar to at least one earlier study[29]. Additionally, there was a significant rise in Hb from the lowest to the second quartile of Zn status although there was no added benefit with higher Zn status. Interestingly, the lowest quartile cut-off value (9·4 μmol/l) was close to the conventional deficiency cut-off. This pattern suggests that a natural saturation level may exist above which the role of Zn in erythropoiesis, or as a cofactor for enzymes that protect erythrocytes from oxidative stress, is minimized.

Both Zn and vitamin B_{12} were positively associated with Hb and positively correlated with each other; however, we found a negative interaction between Zn and vitamin B_{12} and Hb. The mechanism causing these relationships is not understood but perhaps Zn affects vitamin B_{12} metabolism. For example, vitamin B_{12}-dependent methionine synthase and intestinal brush boarder folate conjugase are Zn-dependent enzymes[30,31]. Intakes and bioavailability of vitamin B_{12} and Zn should also be interrelated since vitamin B_{12} and highly bioavailable Zn are found in similar food sources like flesh foods. However, plasma Zn concentration is more affected by recent intake[32] while plasma vitamin B_{12} status reflects longer-term dietary patterns[33], highlighting a continual need for nutrient status indicators that align with duration of exposure.

Vitamin A (retinol) deficiency was relatively uncommon (7·4 %) despite the infrequent intake of plant foods rich in provitamin A. Given the seasonal availability of these foods, it is possible that increased intake of vitamin-A rich foods prior to the food frequency recall period may have impacted the status of this fat-soluble vitamin. In previous studies vitamin A deficiency was found to be consistently associated with anaemia[11], but among our population vitamin A status was not associated with Hb concentration. This finding may be due to the fact that prevalence of vitamin A deficiency was low.

Plasma α-tocopherol status was higher than plasma γ-tocopherol status. This may be due to preferential affinity of tocopherol transfer protein for α-tocopherol, leading to a longer half-life for α-tocopherol compared with γ-tocopherol[34]. Cornwell *et al.*[35] proposed that such preferential retention of α-tocopherol offers an evolutionary advantage by decreasing the availability of γ-tocopherol, which is a probable precursor of mutagenic γ-tocopherol quinine. Nevertheless, it is expected that α- and γ-tocopherol should exhibit similar biological functions, including their influence on Hb, because they are both forms of vitamin E. However, some studies, including the present one, have shown that their effect may differ. Ford *et al.*[36] reported among US adults who did not use supplements that α-tocopherol was not associated with glucose but γ-tocopherol concentration was positively associated with glucose and glycosylated Hb concentration. In another study among British adults, α-tocopherol status was positively associated with healthy food choices, i.e. increased intake of polyunsaturated fats, fresh fruits and fruit juice, and inversely associated with unhealthy food choices, i.e. elevated intake of non-polyunsaturated fats and added sugar; the reverse was observed for γ-tocopherol status[37]. High γ-tocopherol status was also reported to be associated with increased risk of preterm delivery[38]. Along the lines of these contrasting findings, to our knowledge the present study is the first one to report that Hb concentration is negatively associated with γ-tocopherol among pregnant women.

The women may have suffered from other micronutrient deficiencies, including iodine about which we recently reported[39]. However, data on additional important micronutrients, such as other B-vitamins or other tocotrienols, were unavailable, reflecting limitations in resources for biochemical analyses.

Conclusion

Among a population of women in early pregnancy living in rural Bangladesh with apparent Fe sufficiency and minimal rates of inflammation, we found that micronutrient deficiencies were common. However, deficiencies commonly associated with compromised haematopoietic status, including Fe, folate, and vitamin A, were relatively rare. Rather, low Hb was associated both with individual deficiencies of Zn, vitamin B_{12} and α-tocopherol and with the cumulative presence of multiple micronutrient deficiencies. While there are biologically plausible reasons to suggest that these associations could be causal, mechanisms remain to be elucidated. Nevertheless, the observed associations lend further support to the importance of ensuring that pregnant women receive an adequate diet providing a balance of micronutrients, thereby reducing their risk of anaemia and associated health outcomes during this physiologically demanding period.

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Supplementary Materials

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