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Correlates of anaemia in pregnant urban South Indian women: a possible role of dietary intake of nutrients that inhibit iron absorption

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Abstract

Objective: To identify correlates of anaemia during the first trimester of pregnancy among 366 urban South Indian pregnant women.
Design: Cross-sectional study evaluating demographic, socio-economic, anthropometric and dietary intake data on haematological outcomes.
Setting: A government maternity health-care centre catering predominantly to the needs of pregnant women from the lower socio-economic strata of urban Bangalore.
Subjects: Pregnant women (n 366) aged ≥18 and ≤40 years, who registered for antenatal screening at ≤14 weeks of gestation.
Results: Mean age was 22·6 (SD 3·4) years, mean BMI was 20·4 (SD 3·3) kg/m^2 and 236 (64·5 %) of the pregnant women were primiparous. The prevalence of anaemia (Hb <11·0 g/dl) was 30·3 % and of microcytic anaemia (anaemia with mean corpuscular volume <80 fl) 20·2 %. Mean dietary intakes of energy, Ca, Fe and folate were well below the Indian RDA. In multivariable log-binomial regression analysis, anaemia was independently associated with high dietary intakes of Ca (relative risk; 95 % CI: 1·79; 1·16, 2·76) and P (1·96; 1·31, 2·96) and high intake of meat, fish and poultry (1·94; 1·29, 2·91).
Conclusions: Low dietary intake of multiple micronutrients, but higher intakes of nutrients that inhibit Fe absorption such as Ca and P, may help explain high rates of maternal anaemia in India.

Keywords
Anaemia
Pregnancy
Correlates
South India

Anaemia is a global public health problem that affects nearly 2 billion people in both developed and developing countries[1]. Anaemia is highly prevalent among pregnant women in developing countries and is associated with poor pregnancy outcomes[2]. Anaemia in early pregnancy is associated with a 50 % greater risk of inadequate weight gain for gestation[3]. The risk of preterm delivery is increased by 10–40 % with mild anaemia[4]. The National Family Health Survey reported the prevalence of anaemia among pregnant women in India to be 59 %[5]. A survey among pregnant women from sixteen districts of eleven states in India showed that 61·0 % to 96·8 % of pregnant women were anaemic (Hb < 11 g/dl)[6]. Despite the ongoing national anaemia prophylaxis programme of Fe and folic acid supplementation to pregnant women, two large national surveys[5,7] have indicated that there has been little change in the prevalence of anaemia and the adverse consequences associated with it.

Studies conducted in India suggested that micronutrient deficiencies (Fe, folate and vitamin B_{12}) are the primary cause of anaemia in pregnancy[8]. Dietary intake surveys among pregnant women show low intake of several key micronutrients[9] and subclinical deficiencies of riboflavin, pyridoxine and folic acid have been reported[10]. The relative contribution of each of these factors to anaemia in pregnancy often varies by geographical location, season and dietary intake[11]. Even within India there are wide state-level variations in the

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prevalence of moderate and severe anaemia[7,12], often attributed to differences in the simultaneous presence of several micronutrient deficiencies in addition to Fe deficiency, acute and chronic infections and differences in bioavailability of various micronutrients in the diet[13]. Despite a literacy rate of 68·1 % among women in Karnataka[14] and 85·3 % of the women having received antenatal care[15], the prevalence of anaemia among pregnant women in Karnataka remains 47·6 %[16]. We therefore were interested to determine the modifiable risk factors associated with anaemia among pregnant women in urban Karnataka early in their pregnancy.

Materials and methods

Study design

We performed a cross-sectional study among a cohort of pregnant women enrolled in a randomized controlled trial of vitamin B_{12} supplementation (NCT00641862). The study was conducted at Hosahalli Referral Hospital, Bangalore, Karnataka, South India. This government maternity healthcare centre caters predominantly to the needs of pregnant women from the lower socio-economic strata of urban Bangalore and has approximately 200 deliveries per month. Pregnant women were enrolled in early pregnancy (≤14 weeks of gestation) from December 2008 to November 2010. The pregnant women are currently being followed through 2 years postpartum as part of the ongoing trial. The institutional review boards at St John's Medical College Hospital and Harvard School of Public Health approved all study procedures, and written informed consent was obtained from each participant at enrolment.

Study population

All pregnant women aged ≥18 and ≤40 years, who were ≤14 weeks of gestation and registered for antenatal screening at the Hosahalli Referral Hospital, were invited to participate in the study. Pregnant women with multiple gestation, those with a clinical diagnosis of chronic illness (diabetes mellitus, hypertension, heart disease or thyroid disease), those who tested positive for HbSAg (surface antigen of the hepatitis B virus), HIV or syphilis, those who anticipated moving out of the city before delivery, those who were already consuming vitamin B_{12} supplements and those who had been treated for infertility were excluded.

Of the 1376 pregnant women who were contacted at the antenatal clinic during the study period, 958 were excluded for the following reasons: planning to deliver outside Bangalore at maternal home town (*n* 836); wishing to terminate the pregnancy (*n* 67); age <18 years (*n* 4); history of hypertension (*n* 7); previous Caesarean section (*n* 4); and pregnancy was not confirmed (*n* 40). Of the remaining 418 pregnant women who were considered eligible fifty-two declined to participate, leaving 366 who consented.

Sociodemographic and anthropometric information

The pregnant women were interviewed by trained research assistants to obtain sociodemographic information. Gestational age (in weeks) was calculated from the reported first day of the last menstrual period. A digital balance (Salter's 9016, Tonbridge, Kent, UK) was used to record the weights of all mothers to the nearest 100 g. Measurements of height were made using a stadiometer to the nearest 0·1 cm. Mid-upper arm circumference was measured to the nearest 0·1 cm using a plastic measuring tape and triceps skinfold was measured using a Holtain calliper (Crosswell, Crymych, UK). BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2).

Haematological data

Blood was drawn from pregnant women after an overnight fast by venepuncture and collected in both EDTA-containing and plain Vacutainer™ tubes (BD Diagnostics, Franklin Lakes, NJ, USA). Hb and complete blood count were measured on an automated Coulter counter (ABX Pentra 60C+; Horriba Ltd, Kyoto, Japan). Anaemia was defined as Hb < 11·0 g/dl and severe anaemia as Hb < 7·0 g/dl[1]. Microcytosis was defined as mean corpuscular volume (MCV) < 80 fl[17], while microcytic anaemia was defined as anaemia with MCV < 80 fl. Haematocrit was considered to be low in pregnancy at values <33 %[18] while the reference range for red-cell distribution width was considered to be 11·5 % to 14·5 %[17].

Dietary data

A pre-tested interviewer-administered FFQ was used to assess the habitual dietary intake for the three months preceding the date of the pregnant women's enrolment into the study. Standard measures were placed before the respondent to quantify the portion size of each food item when administering the questionnaire. The questionnaire was adapted from a questionnaire developed for the urban population residing in South India[19] and has a food list of 127 items, derived from a food database developed from studies at St John's Medical College. Nutrient scores were computed by multiplying the relative frequency of consumption of each food item by the nutrient content of the standard portion size. Nutrient information was obtained on twenty-seven macro- and micronutrients. Energy-adjusted nutrient intakes were calculated by the residual method[20]. Data on the amount of food groups consumed were calculated as the total grams of the food groups consumed daily.

Statistical analysis

Continuous data were summarised as means and standard deviations, and categorical data as numbers and percentages.

Women who had missing data on any variable were not considered for the analysis involving that variable. The association of anaemia with maternal sociodemographic characteristics and energy-adjusted nutrient intakes, categorized by tertiles, was examined using the χ^2 test. The parameters that were found significant in this analysis were considered in multivariable log-binomial regression analysis to examine the independent effect of certain dietary nutrient intakes and food group intakes while adjusting for possible confounding effects of maternal sociodemographic characteristics. All maternal sociodemographic characteristics with $P<0\cdot20$ in the univariate analyses were considered in the multivariable model. Characteristics contributing a >5 % change in the adjusted estimate after exclusion of the covariate from the full model were retained as confounders in the final model. Separate models were constructed for Ca and P as they were collinear and could not be considered in the same model. Similar analyses were performed with tertiles of intakes of certain food groups which were likely to be associated with anaemia. In addition, Fe intake was retained in the model as an exposure of interest. The presence of effect modification by maternal sociodemographic characteristics, dietary intake of nutrients and food groups was examined using stratified analyses. No effect modification was observed (data not shown). Relative risks (RR) with 95 % confidence intervals and corresponding *P* values for both unadjusted and adjusted models are presented. Statistical analyses were carried out with the SPSS statistical software package version 16 (SPSS Inc., Chicago, IL, USA). Log-binomial regression analysis was carried out using the PROC GENMOD program in the SAS statistical software package version 9·2 (SAS Institute, Cary, NC, USA).

Table 1 Baseline sociodemographic and anthropometric characteristics of the study population: pregnant Indian women aged ≥18 and ≤40 years (*n* 366), enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010

Parameter	*n*	%
Age (years) (*n* 366)		
<20	102	27·9
20–24	170	46·4
25–29	77	21·0
≥30	17	4·6
Level of education (*n* 364)		
No formal education	15	4·1
Finished high school (10th grade)	257	70·5
Post high school	71	19·5
University degree and above	21	5·8
Occupation (*n* 364)		
Unemployed	305	83·8
Unskilled worker	21	5·8
Skilled worker	27	7·4
Others (secretarial jobs, teachers, business, shop owners)	11	3·1
Parity (*n* 366)		
0	236	64·5
≥1	130	35·6
BMI<18·5 kg/m^2 (*n* 364)	114	31·3
	Mean	SD
Age (years) (*n* 366)	22·6	3·4
Gestational age at recruitment by LMP (weeks) (*n* 366)	11·2	2·4
Anthropometry (*n* 364)		
Weight (kg)	47·8	8·1
Height (cm)	153·0	5·6
BMI (kg/m^2)	20·4	3·3
Mid-upper arm circumference (cm)	23·6	3·1
Triceps skinfold (mm)	15·6	5·7
	Median	25th, 75th percentile
Total monthly household income (Rs)* (*n* 365)	6000	4500, 9000

LMP, last menstrual period; Rs, rupees.
*$US 1 = Rs 50·2 as of 21 January 2012.

Results

The mean age of the pregnant women was 22·6 (SD 3·4) years and the mean gestational age was 11·2 (SD 2·4) weeks (Table 1). High-school graduation was attained by at least 95·8 % of the women. A majority of them (83·8 %) were unemployed. Primiparous pregnant women made up 64·5 % of the cohort. The mean weight and height of the women were 47·8 (SD 8·1) kg and 153·0 (SD 5·6) cm, respectively. BMI was less than 18·5 kg/m^2 in 31·3 % of the women[21]. The mean mid-upper arm circumference (an estimate of peripheral muscle and fat mass) and triceps skinfold (an estimate of peripheral fat mass) of the women were 23·6 (SD 3·1) cm and 15·6 (SD 5·7) mm, respectively. None of the women were consuming Fe or folate supplements on study entry.

The mean Hb and haematocrit levels on study entry were 11·5 (SD 1·5) g/dl and 34·6 (SD 5·1) %, respectively (Table 2). The prevalence of anaemia (Hb < 11·0 g/dl) at baseline was 30·3 % (111/366). Very few women (0·8 %) had severe anaemia (Hb < 7·0 g/dl). Nearly one-third of the women had microcytosis (MCV <80 fl) and 20·2 %

Table 2 Haematological characteristics of the study population: pregnant Indian women aged ≥18 and ≤40 years (*n* 366), enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010

Parameter	Mean	SD
Hb (g/dl)	11·5	1·5
Haematocrit (%)	34·6	5·1
MCV (fl)	81·1	8·9
RDW (%)	13·3	2·2
	n	%
Anaemia (Hb < 11·0 g/dl)	111	30·3
Hb < 8·5 g/dl	18	4·9
MCV < 80 fl	117	32·0
MCV > 90 fl	37	10·1
RDW > 14·5 %	81	22·1
Anaemia and MCV < 80 fl	74	20·2
Anaemia and MCV > 90 fl	2	0·5

MCV, mean corpuscular volume; RDW, red-cell distribution width.

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	Mean (SD) Anaemic	Mean (SD) Non-anaemic
Energy (kJ/d)	7513 (1565)	7162 (1963)
Protein (g/d)	55·0 (14·1)	50·4 (14·0)
Fat (g/d)	49·6 (14·1)	46·4 (14·0)
Vitamin C (mg/d)	102 (32)	107 (51)
Folate (μg/d)	283 (272)	279 (96)
Vitamin B_{12} (μg/d)	1·96 (2·33)	1·92 (2·80)
Ca (mg/d)	895 (262)	797 (291)
Fe (mg/d)	15·6 (3·9)	14·9 (5·2)
Cu (mg/d)	2·19 (0·66)	2·06 (0·77)
Zn (mg/d)	8·05 (1·94)	7·39 (2·18)
Mn (mg/d)	6·9 (3·1)	6·0 (3·2)
P (g/d)	1·26 (3·4)	1·16 (2·8)

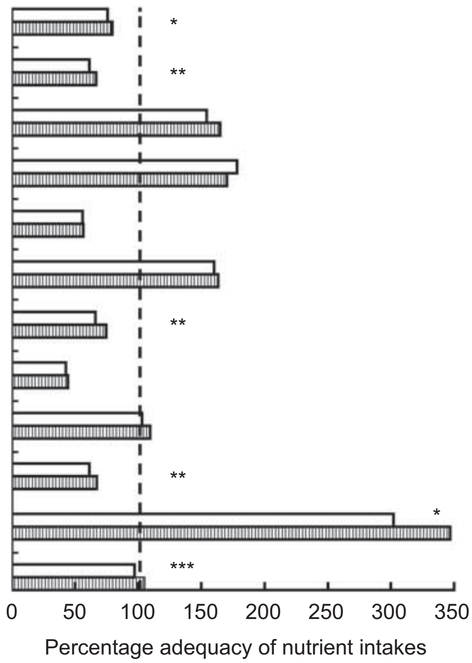

Fig. 1 Dietary intakes, means with standard deviations (left) and as percentage adequacy of the corresponding Indian RDA (right), among anaemic (▥, *n* 110) and non-anaemic (□, *n* 252) pregnant Indian women aged ≥18 and ≤40 years, enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010. Mean values were significantly different between anaemic and non-anaemic pregnant women using the independent *t* test (two-sided): $^*P < 0·05$, $^{**}P < 0·01$, $^{***}P < 0·001$

had anaemia with microcytosis. The percentage of women with a red-cell distribution width >14·5 %, suggestive of Fe-deficiency anaemia, was 22·1 %.

The dietary intakes of the anaemic and non-anaemic pregnant women, as means with standard deviations and as percentage adequacy compared with the Indian RDA, are presented in Fig. 1. The mean Fe intakes of anaemic (15·6 mg/d) and non-anaemic women (14·9 mg/d) were not significantly different from each other; however, intakes were very low and met only 44·5 % and 42·6 % of the RDA, respectively, in the two groups. Although the overall mean energy intake was low among both anaemic (7513 kJ/d) and non-anaemic women (7162 kJ/d), it was significantly higher among pregnant women with anaemia compared with those without anaemia ($P < 0·05$). Protein intake met between only 60 % and 70 % of the RDA, although it was significantly higher among anaemic pregnant women ($P < 0·01$). The percentage adequacy of fat intake was greater than 100 % in comparison to the RDA among both anaemic and non-anaemic pregnant women. Mean dietary intake of Ca was 895 mg/d among anaemic pregnant women compared with 797 mg/d among pregnant women without anaemia ($P < 0·01$). The dietary intakes of other nutrients known to have an inhibitory effect on Fe absorption, such as P, Mn and Zn, were significantly higher among the anaemic pregnant women than those without anaemia ($P < 0·001$, $P = 0·03$ and $P = 0·002$, respectively). The primary food groups contributing towards higher intake of P in anaemic pregnant women were cereals, pulses and milk and milk products; mean P intakes from these were 0·41 (SD 0·13) g/d, 0·11 (SD 0·07) g/d and 0·25 (SD 0·23) g/d, respectively. The main contributors towards high Mn intake in anaemic pregnant women were cereals and tea, mean Mn intakes from these being 2·6 (SD 0·8) mg/d and 4·2 (SD 2·3) mg/d, respectively. The significant difference in intakes of protein, Ca, P, Mn and Zn between anaemic and non-anaemic pregnant women persisted even after energy adjustment of nutrients. The intakes of vitamin C, vitamin B_{12} and Cu were all greater than the RDA and were not significantly different between the two groups. The primary food groups contributing towards higher intake of vitamin C were vegetables and fruits, with mean vitamin C intakes of 40·4 (SD 22·5) and 23·0 (SD 26·7) mg/d, respectively. The main contributor to Cu intake was cereals (mean 0·84 (SD 0·33) mg/d); while milk and meat contributed towards vitamin B_{12} intake, mean intakes from the latter being 0·40 (SD 0·40) and 0·36 (SD 0·38) μg/d, respectively.

Results of log-binomial regression analyses of anaemia are presented in Table 3. Two separate multivariable models are presented: Model 1 (sociodemographic factors and energy-adjusted micronutrient intakes) and Model 2 (sociodemographic factors and food dietary intakes from

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Table 3 Sociodemographic and nutritional correlates of anaemia among the study population: pregnant Indian women aged ≥18 and ≤40 years (*n* 366), enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010

		Univariate analyses			Multivariable Model 1* (*n* 358)			Multivariable Model 2† (*n* 358)		
Parameter	*n*/*N*	RR	95 % CI	*P* value	Adjusted RR	95 %CI‡	*P* value	Adjusted RR	95 %CI‡	*P* value
Occupation										
Working at home	86/305	1·00	Ref.	0·016	1·00	Ref.	0·06	1·00	Ref.	0·05
Working outside the home	25/58	1·53	1·09, 2·16		1·45	1·01, 2·09		1·40	1·01, 1·94	
Monthly family income										
Tertile 1 (<Rs 5000)	43/155	1·00	Ref.	0·15	1·00	Ref.	0·14	1·00	Ref.	0·220
Tertile 2 (Rs 5000–8000)	26/98	0·74	0·52, 1·05		0·71	0·50, 1·02		0·76	0·55, 1·05	
Tertile 3 (>Rs 8000)	42/112	0·71	0·47, 1·06		0·73	0·49, 1·08		0·79	0·53, 1·17	
Education										
Less than high school	37/95	1·43	0·94, 2·18	0·12	1·36	0·89, 2·09	0·24	1·49	0·98, 2·26	0·11
High school	49/177	1·02	0·67, 1·54		1·04	0·68, 1·57		1·10	0·73, 1·64	
More than high school	25/92	1·00	Ref.		1·00	Ref.		1·00	Ref.	
Fe intake (mg/d)										
Tertile 1 (<13·9)	32/120	0·83	0·56, 1·23	0·55	0·76	0·51, 1·14	0·41	–		–
Tertile 2 (13·9–15·6)	39/121	1·00	0·69, 1·44		0·89	0·62, 1·28		–		
Tertile 3 (>15·6)	39/121	1·00	Ref.		1·00	Ref.		–		
Folate intake (μg/d)										
Tertile 1 (<260·6)	42/120	1·46	0·98, 2·18	0·15	1·36	0·89, 2·07	0·34	–		–
Tertile 2 (260·6–293·5)	39/121	1·34	0·89, 2·02		1·25	0·83, 1·89		–		
Tertile 3 (>293·5)	29/121	1·00	Ref.		1·00	Ref.		–		
Ca intake (mg/d)										
Tertile 1 (<738·7)	24/121	1·00	Ref.	0·006	1·00	Ref.	0·02	–		–
Tertile 2 (738·7–887·7)	41/121	1·71	1·10, 2·64		1·59	1·04, 2·42		–		
Tertile 3 (>887·7)	45/120	1·89	1·23, 2·90		1·79	1·16, 2·76		–		
P intake (mg/d)§										
Tertile 1 (<1147·1)	25/121	1·00	Ref.	<0·001	1·00	Ref.	0·002	–		–
Tertile 2 (1147·1–1234·3)	34/121	1·36	0·87, 2·13		1·28	0·82, 1·98		–		
Tertile 3 (>1243·3)	51/120	2·06	1·37, 3·09		1·96	1·30, 2·96		–		
Fibre intake (g/d)										
Tertile 1 (<4·1)	30/121	1·00	Ref.	0·15	–		–	1·00	Ref.	0·11
Tertile 2 (4·1–6·2)	36/120	1·21	0·80, 1·83		–		–	1·18	0·80, 1·74	
Tertile 3 (>6·2)	44/121	1·47	0·99, 2·17		–		–	1·46	1·01, 2·11	
Meat, poultry & fish intake (g/d)										
Tertile 1 (<9·2)	24/121	1·00	Ref.	0·002	–		–	1·00	Ref.	0·002
Tertile 2 (9·2–25·6)	37/123	1·52	0·97, 2·37		–		–	1·35	0·87, 2·10	
Tertile 3 (>25·6)	49/118	2·09	1·38, 3·18		–		–	1·94	1·29, 2·91	

RR, relative risk; Ref. referent category.

P values (two-sided) are reported for the adjusted RR.

*Multivariable log-binomial regression of anaemia *v.* occupation, monthly family income, education, Fe, Ca, folate and P intakes.

†Multivariable log-binomial regression of anaemia *v.* occupation, monthly family income, education, fibre and meat, poultry & fish intakes.

‡Adjusted RR from a log-binomial regression model adjusted for the effects of the other variables in the model.

§Due to collinearity of Ca and P intakes ($r = 0{\cdot}8$), the adjusted RR for dietary P intakes were obtained from a separate model adjusted for the effects of the other variables in the model.

food groups). In the univariate analyses, the socio-demographic variable that was significantly associated with a higher prevalence of anaemia was working outside the home ($P=0{\cdot}016$). Maternal age, parity, income and education were not associated with anaemia. Energy-adjusted nutrient intakes associated with higher prevalence of anaemia included higher intake of Ca and P ($P=0{\cdot}006$ and $P<0{\cdot}001$, respectively). In multivariable Model 1, working outside the home was no longer associated with anaemia. The significant association of Ca and P intakes with anaemia observed in the univariate analyses persisted in the multivariable Model 1 (Table 3).

In multivariable Model 2 (Table 3), the RR of anaemia was higher for pregnant women with the greatest intakes of meat, fish and poultry (RR = 1·94; 95 % CI 1·29, 2·91), consistent with the univariate analyses. The relationship of milk intake with anaemia did not show a monotonic trend and was not significant in either the univariate or multivariable analyses.

Discussion

In a cross-sectional analysis of 366 urban pregnant Indian women, we found a high prevalence of both anaemia (30·3 %) and microcytic anaemia (20·2 %) during the first trimester of pregnancy. Among these pregnant women, who had low intakes of Fe and several other nutrients, higher intakes of Ca and P (dietary components known to inhibit Fe absorption) were independently associated with a higher prevalence of anaemia.

Our study is unique in that we have identified the prevalence and dietary correlates of anaemia as early as ≤14 weeks of gestation. A study from the urban areas of Udipi, Karnataka found the prevalence of anaemia to be 50·1 % (Hb determined by the cyanmethaemoglobin method) among pregnant women at ≤14 weeks of gestation, and identified age, parity, education, socio-economic status, compliance with Fe supplements, history of bleeding and food selection ability as significant determinants of anaemia; however, dietary factors were not studied(22). Other studies have reported a higher prevalence of anaemia than our reported rate of 30·3 % but these have been carried out among pregnant women later in pregnancy and have used different methods of measuring Hb(23–25). The present anaemia prevalence is also lower than the published figures of 59 % for any anaemia among pregnant Indian women(5) and 32 % for moderate to severe anaemia among pregnant women in Karnataka(7). This may be attributed to the fact that our study was a hospital-based study recruiting urban women in early pregnancy based on strict inclusion and exclusion criteria, and possible differences in the method of Hb analysis. It is also noteworthy that the years of schooling attained by pregnant women in our cohort were generally higher in comparison to the overall level of education attainment by pregnant women from urban Karnataka(26). Nevertheless, a prevalence of 30·3 % anaemia is in itself an indication that anaemia continues to be a problem of public health significance among pregnant Indian women.

Our results indicate that nearly one-third of the pregnant women had BMI $<18{\cdot}5\,kg/m^2$. This is in accordance with nationally representative data from the National Family Health Survey that has shown more than one-third of women to have a BMI $<18{\cdot}5\,kg/m^2$, reflecting chronic energy and micronutrient deficiencies(27). The mean intakes of several macro- and micronutrients were well below the RDA for both pregnant anaemic and non-anaemic women. For example, the energy intake was low in comparison to the RDA among all pregnant women in our cohort, although it was higher among the anaemic pregnant women compared with the non-anaemic pregnant women possibly reflecting a diet high in energy but poor in quality. Similar low intake of energy has been reported among pregnant women from urban South India during early pregnancy(28). The mean dietary intake of Fe was less than 50 % of the RDA. It is noteworthy that all of the pregnant women were ≤14 weeks of gestational age and at enrolment none was receiving Fe or folic acid supplements. Perhaps because of the low and narrow range of dietary Fe intake, we did not observe any association of Fe intake with anaemia. These results are consistent with data from the National Nutrition Monitoring Bureau surveys in India that also showed no correlation of Fe intake with anaemia(13). Vitamin C is known to have an enhancing and dose-dependent effect on Fe absorption in many single-meal radioisotope studies in human volunteers(29), and can overcome the negative effect of inhibitors on Fe absorption(30). However prolonged cooking and storage of food can reduce its enhancing effect(31). Although the mean intake of vitamin C among pregnant women in our cohort exceeded the RDA, it was apparently not sufficient to overcome the inhibitory effect of Fe absorption inhibitors such as P and Ca. This may be due to practices commonly observed among these women, such as prolonged cooking and allowing the meals to stand for longer durations. On the contrary, intakes of Fe absorption inhibitors such as Mn, P and Zn were higher in anaemic pregnant women.

In the setting of low dietary Fe intake, the intakes of several other nutrients and their role in increasing the risk of anaemia through possible interactions with Fe are important to consider. A higher intake of Mn may impose a risk for reduced Fe utilization by affecting Fe absorption, while higher doses of Zn in aqueous solutions are known to impair Fe absorption, although this effect has not been observed when Zn was added to meals(32). Among pregnant women we observed that higher intake of Ca was associated with a greater risk of anaemia. In single-meal studies conducted in adult men and women, $CaCl_2$ at doses ranging from 40 to 300 mg had a dose-dependent inhibitory effect on the absorption of 5 mg

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non-haem Fe, and 165 mg of Ca given as milk, cheese or $CaCl_2$ diminished absorption of 5 mg haem Fe[33,34]. More recently, an isolated effect of $CaCl_2$ on absorption of both 5 mg non-haem Fe and haem Fe was observed at Ca doses ≥1000 mg and 800 mg, respectively, among non-pregnant women[35]. The effect of Ca supplementation on the uptake of haem Fe is independent of Fe bioavailability in the meal[36] and is a direct effect on haem Fe absorption rather than an indirect counteracting effect on the enhancing effect of meat[37]. This effect is not only seen when Ca and Fe are taken together in the same meal[38,39]. Ca is known to inhibit Fe absorption by the formation of poorly soluble calcium phytate complexes[40] or at the cellular level by its effect on DMT 1 (divalent metal-ion transporter-1) receptors[41]. The competitive inhibition between Ca and Fe in the final transport steps from the mucosal cells to the plasma, common for haem and non-haem Fe, has also been suggested[33]. This significant association of dietary Ca intake with anaemia may have a greater relevance among pregnant women from India because of their inadequate Fe stores, overall low intakes of both Fe and Ca, a predominantly cereal/pulse-based diet and the relative low Fe bioavailability of Indian meals.

Higher intake of P was also observed to be an independent risk factor for anaemia among pregnant women in the present study. P may have a direct inhibitory effect on Fe absorption[42]. Even relatively small quantities of residual phytate, which is the storage form of P (<10 mg phytic acid/meal), are known to strongly inhibit Fe absorption[43]. Although we did not analyse the phytate content in the meals of women in this current cohort, results from another study in a similar group of young women of low socioeconomic status from Bangalore showed that the phytate content in their diets was as high as 1287 mg/d[44]. We also observed that a higher intake of meat was associated with a greater risk of anaemia. It is, however, noteworthy that the median (25th, 75th percentile) intake of meat even in the highest group of meat intake was only 39 (32, 50) g/d, making it difficult to draw any conclusions about the relationship between meat intake and anaemia.

In addition to the nutritional factors that cause anaemia, chronic Fe losses due to parasitic infections such as hookworm and schistosomiasis may cause anaemia[44]. We did not evaluate for the presence of parasitic infections among pregnant women in our cohort; however, a low prevalence of parasitic infections has been previously reported among women from urban slums in South India[44]. Our study is limited in that the dietary data were recorded with an FFQ and not via alternative methods including prospective food weighing and other techniques. However, we did employ a validated FFQ with a trained interviewer and portion sizes to minimize recall bias. Fluctuations in appetite and nausea commonly experienced by pregnant women in the first trimester may have influenced reported dietary intakes[45]. Further, our assessment of Fe status was limited to red-blood-cell morphologic indices. The results of our study are based on cross-sectional data with a moderate sample size, so caution should be exercised when drawing conclusions, especially for different populations. In addition, our study results are based on a sample of pregnant women enrolled in a clinical trial at a single hospital and therefore the findings may not be representative of all urban Indian pregnant women.

Conclusions

The results of the present study indicate that higher intakes of Ca and P may be important correlates of anaemia among women with low Fe intake. Further research is warranted to elucidate the modulatory effect of Ca and P on Fe absorption and the levels at which these effects are pronounced among pregnant women in the Indian subcontinent, where such effects have not been previously documented.

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