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ORIGINAL ARTICLE

Vitamin B_{12} status in pregnant women and their infants in South India

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BACKGROUND/OBJECTIVES: Vitamin B_{12} deficiency during pregnancy has been associated with increased risk of adverse perinatal outcomes. However, few studies have investigated the burden and determinants of vitamin B_{12} status in young infants. This study was conducted to determine the associations between maternal and infant vitamin B_{12} status.

SUBJECTS/METHODS: Pregnant women participating in a vitamin B_{12} supplementation trial in Bangalore, India, were randomized to receive vitamin B_{12} (50 μg) or placebo supplementation daily during pregnancy through 6 weeks postpartum. All women received 60 mg of iron and 500 μg of folic acid daily during pregnancy, as per standard of care. This prospective analysis was conducted to determine the associations between maternal vitamin B_{12} biomarkers (that is, plasma vitamin B_{12}, methylmalonic acid (MMA) and tHcy) during each trimester with infant vitamin B_{12} status ($n = 77$) at 6 weeks of age.

RESULTS: At baseline (⩽14 weeks of gestation), 51% of mothers were vitamin B_{12} deficient (vitamin $B_{12} < 150$ pmol/l) and 43% had impaired vitamin B_{12} status (vitamin $B_{12} < 150$ pmol/l and MMA > 0.26 μmol/l); 44% of infants were vitamin B_{12} deficient at 6 weeks of age. After adjusting for vitamin B_{12} supplementation, higher vitamin B_{12} concentrations in each trimester were associated with increased infant vitamin B_{12} concentrations and lower risk of vitamin B_{12} deficiency in infants ($P < 0.05$). After adjusting for vitamin B_{12} supplementation, infants born to women with vitamin B_{12} deficiency had a twofold greater risk of vitamin B_{12} deficiency ($P < 0.01$). Higher maternal folate concentrations also predicted lower risk of vitamin B_{12} deficiency in infants ($P < 0.05$). Impaired maternal vitamin B_{12} status, which combined both circulating and functional biomarkers, was the single best predictor of infant vitamin B_{12} status.

CONCLUSIONS: Impaired maternal vitamin B_{12} status throughout pregnancy predicted higher risk of vitamin B_{12} deficiency in infants, after adjusting for vitamin B_{12} supplementation. Future interventions are needed to improve vitamin B_{12} status periconceptionally, and to ensure optimal vitamin B_{12} status and health outcomes in pregnant women and their children.

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INTRODUCTION

Vitamin B_{12} deficiency is a major threat to public health globally.[1,2] The prevalence of vitamin B_{12} deficiency is highest in resource-limited settings, including South Asia.[3–8] Vitamin B_{12} is obtained in the diet through consumption of animal products, including meat, poultry, fish, eggs and dairy. Several studies have reported low vitamin B_{12} status in vegan or vegetarian individuals and in low- and middle-income settings, particularly in populations with low intake of animal source foods.[9,10] In particular, the burden of vitamin B_{12} deficiency in India is thought to be among the highest in the world.[1]

Maternal vitamin B_{12} deficiency has been associated with greater risk of pregnancy complications, such as spontaneous abortion, low birth weight, intrauterine growth restriction and neural tube defects.[11] Children born to women with vitamin B_{12} deficiency have an increased risk of adverse health outcomes, including deficits in growth and development and anemia.[12–14] In the parent-randomized trial in Bangalore, India, daily maternal vitamin B_{12} supplementation (50 μg/day) with iron and folic acid during pregnancy through 6 weeks postpartum significantly improved maternal vitamin B_{12} status ($P < 0.01$), breast milk ($P < 0.01$) and infant ($P < 0.01$) vitamin B_{12} concentrations, compared to iron-folic acid alone.[15]

Previous studies in Turkey, Germany, Norway and Brazil have reported associations between maternal and infant vitamin B_{12} status at birth.[15–18] However, few prospective studies have been conducted to examine the burden and determinants of vitamin B_{12} status in young infants, and there is limited data from India.

In the parent-randomized trial in Bangalore, India, pregnant women were randomized to daily maternal vitamin B_{12} supplementation with iron and folic acid during pregnancy through 6 weeks postpartum, compared to iron-folic acid alone, to determine the effects on maternal, breast milk and infant vitamin B_{12} concentrations.[15] We conducted this prospective analysis among 77 mother–infant pairs who were participating in this randomized trial to: (1) determine the prevalence and determinants of inadequate vitamin B_{12} status during pregnancy and early childhood; and (2) examine the associations of maternal vitamin B_{12} biomarkers at each trimester with infant outcomes at 6 weeks

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of age, including vitamin B_{12}, vitamin B_{12} deficiency, methylmalonic acid and homocysteine concentrations.

MATERIALS AND METHODS

Study population

Participants were pregnant women who were enrolled in a randomized, double-blind, placebo-controlled trial of vitamin B_{12} supplementation in Bangalore, India. This trial was conducted to examine the effects of daily prenatal vitamin B_{12} supplementation on biomarkers of maternal vitamin B_{12} status during pregnancy. The detailed design of the study has been previously described.[15] Briefly, pregnant women were recruited from Hosahalli Referral Hospital in Bangalore, India, and randomized to receive vitamin B_{12} supplementation (50 μg/day) or placebo daily during pregnancy through 6 weeks postpartum. All women received 60 mg of iron and 500 μg of folic acid supplementation daily beginning at their first prenatal visit, as per standard of care.

Pregnant women were eligible for the study if they were at least 18 years of age, $\leqslant$ 14 weeks of gestation at enrollment, healthy and carrying a single fetus. Women were excluded if they had any known medical complications, including HIV infection, hepatitis B or syphilis. Women with serious pre-existing medical conditions, previous cesarean section, or who were taking daily vitamin supplements in addition to iron-folate were also excluded. A flow chart of participants in this study is presented in Figure 1.

Ethics

The research protocols and study procedures were approved by the Institutional Ethical Board of St John's Medical College and the TH Chan Harvard School of Public Health Human Subjects Committee. Written informed consent was obtained from all participants. A Data Safety and Monitoring Board met twice annually during the course of the trial.

Follow-up procedures

Structured interviews were conducted to collect information on socio-demographic characteristics, including maternal age, educational level, socioeconomic status and obstetric history. A clinical examination was conducted including vital signs and blood pressure, and obstetric, reproductive and neurological examinations were conducted. Detailed clinical, socio-demographic and anthropometric data were collected prospectively. Maternal weight was recorded using a digital balance to the nearest 100 g; height was measured using a stadiometer to the nearest 0.1 cm; and mid-upper arm circumference, and triceps, biceps and subscapular skinfold thickness measurements were measured in triplicate by trained research assistants.

Laboratory investigations: blood sample collection

Maternal blood samples were collected at study visits during each of the three trimesters, or early ($<$ 14 weeks gestation), mid- (24 weeks), and late- (34 weeks) gestation (that is, median (interquartile range (IQR)); T1: 10.6 (9.1, 12.6); T2: 24.1 (23.7, 25.0); T3: 33.1 (32.7, 33.6) weeks, respectively), and infant blood samples were collected at 6 weeks of age by venipuncture. The laboratory procedures and biochemical analyses in this trial have previously been described.[15] Briefly, approximately 10 ml of blood was collected from mothers during pregnancy and their infants at 6 weeks of age by venipuncture in both EDTA and plain vacutainer tubes (BD Biosciences, Haryana, India) and stored on ice until centrifugation ($<$ 4 h). Whole blood samples were analyzed for hemoglobin and complete blood count, using an automated Coulter counter (ABX Pentra C+; Horiba Medical, New Delhi, India). Plasma and red blood cells were separated and stored at or below −80 °C until analysis for plasma vitamin B_{12}, homocysteine, methylmalonic acid and erythrocyte folate concentrations.

Biomarkers of vitamin B_{12} status

Plasma vitamin B_{12} was measured via electrochemiluminescence (Elecsys 2010, Roche Diagnostics, Mannheim, Germany). The intraday and interday assay CVs for plasma vitamin B_{12} were 0.54 and 2.44%, respectively. Plasma

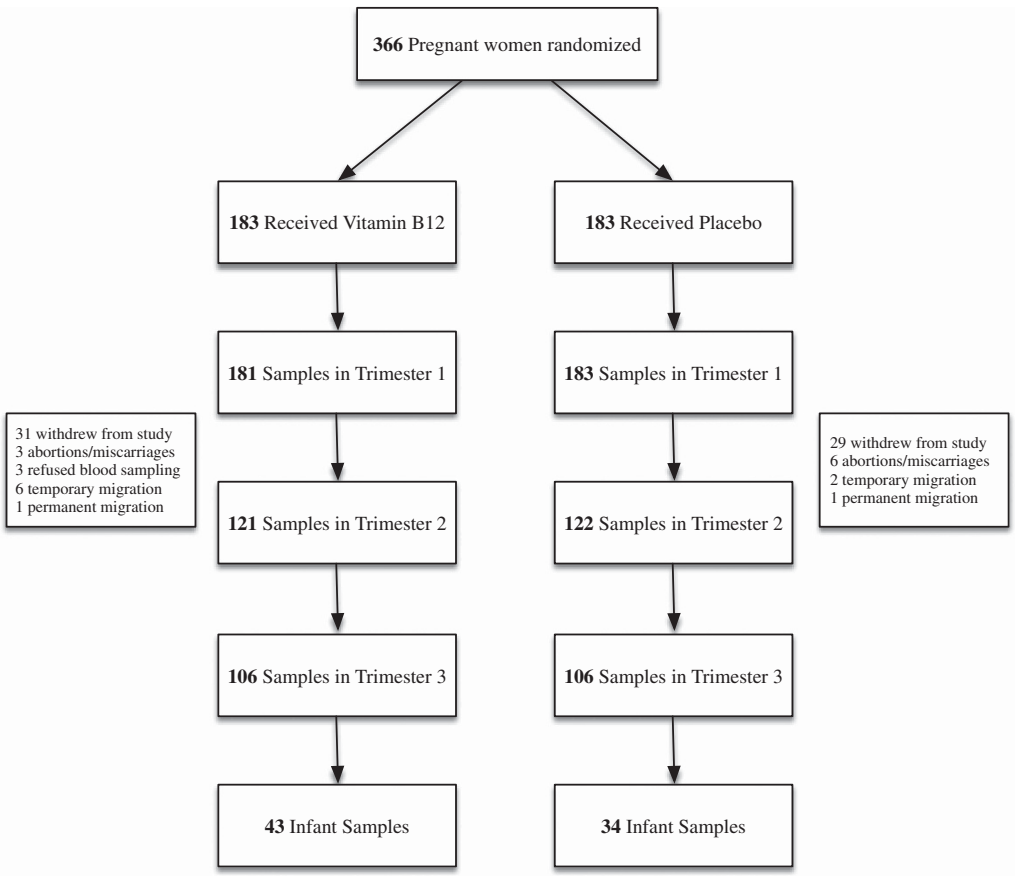

Figure 1. Summary of enrollment and analysis of samples.

Table 1. Characteristics of the study population

Maternal characteristics[a]	*Entire cohort* (n = *366)*	*Current study* (n = *77)*
Vitamin B_{12} intervention, *n* (%)	183 (50)	43 (56)
Socio-demographic		
Age, years	22 (20, 24)	23 (20, 25)
Monthly household income, INR[b]	6000 (4500, 9000)	8000 (5000, 10 000)
< 6000 INR, *n* (%)	161 (44)	26 (34)
Standard of living index		
0–22, *n* (%)	127 (35)	20 (26)
23–28, *n* (%)	124 (34)	28 (36)
29–64, *n* (%)	115 (31)	29 (38)
Gestational age at randomization, weeks	11.4 (9.6, 13.3)	11.0 (9.3, 12.7)
Parity		
Nulliparous, *n* (%)	236 (64)	48 (62)
Primiparous or multiparous, *n* (%)	130 (36)	29 (38)
Anthropometric		
Weight, kg	46.7 (41.6, 53.0)	46.2 (40.8, 53.0)
Height, cm	153 (149, 157)	152 (149, 157)
< 150 cm, *n* (%)	102 (28)	25 (32)
Body mass index, kg/m^2	19.6 (18.1, 22.5)	20.0 (17.9, 22.1)
< 18.5 kg/m^2, *n* (%)	114 (31)	24 (31)
Mid-upper arm circumference, cm	23.0 (21.5, 25.5)	23.0 (21.5, 26.0)
Biochemical		
Hemoglobin, g/dl	11.7 (10.8, 12.6)	11.8 (10.6, 12.7)
< 11.0 g/dl, *n* (%)	109 (30)	22 (29)
Hematocrit, %	35.0 (32.4, 37.4)	35.1 (32.5, 37.7)
Mean corpuscular volume, fL	83 (78, 87)	84 (79, 89)
Plasma vitamin B_{12}, pmol/l	149 (110, 204)	150 (103, 187)
< 150 pmol/l, *n* (%)	180 (51)	36 (50)
Plasma MMA, μmol/l	0.47 (0.28, 0.67)	0.50 (0.31, 0.67)
> 0.26 μmol/l, *n* (%)	273 (76)	58 (76)
Plasma tHcy, μmol/l	9.23 (5.75, 10.08)	9.06 (6.44, 12.91)
> 15.0 μmol/l, *n* (%)	91 (25)	13 (17)
Impaired vitamin B_{12} status[c]	149 (43)	31 (43)
cB12[d]	−0.79 (−1.26, −0.18)	−0.80 (−1.29, −0.15)
Elevated vitamin B_{12}, *n* (%)	3 (1)	2 (3)
Adequate vitamin B_{12}, *n* (%)$_2$	127 (36)	24 (33)
Decreased vitamin B_{12}, *n* (%)	169 (48)	38 (53)
Possibly deficient, *n* (%)	49 (14)	8 (11)
Probably deficient, *n* (%)	1 (< 1)	0 (0)
Erythrocyte folate, nmol/l	387 (291, 496)	399 (290, 487)
< 340 nmol/l, *n* (%)	136 (38)	29 (38)

Abbreviations: INR, Indian rupees; IQR, interquartile range; MMA, methylmalonic acid; tHcy, total homocysteine. [a]Values are median (IQR) and *n* (%). [b]100 INR was equivalent to approximately USD$2 at the time the study was conducted. [c]Impaired vitamin B_{12} status: plasma vitamin B_{12} < 150 pmol/l plus MMA > 0.26 μmol/l. [d]cB12, a combined indicator of vitamin B_{12} status modified for three biomarkers (vitamin B_{12}, MMA and tHcy), was calculated using the method developed by Fedosov *et al*.[20]

methylmalonic acid and tHcy were assessed by gas chromatography-mass spectrometry (Varian 3800, Palo Alto, CA, USA).[16] The intraday assay CVs for plasma methylmalonic acid (MMA) and tHcy were 6.92 and 5.60%, and the interday assay CVs were 5.57% and 5.04%, respectively. Erythrocyte folate concentrations were determined by a competitive immunoassay with direct chemiluminescence detection on an automatized immunoanalyzer (ADVIA Centaurs, Bayer Health Care Diagnostics, Tarrytown, NY, USA),[17] with intra-assay and interassay variabilities of 1.9 and 5.2%, respectively. The folate concentrations in the hemolysate were converted to whole blood values by adjusting for hematocrit. The laboratory procedures and biochemical analyses are described in further detail in the primary randomized trial.[15]

Statistical analyses

Vitamin B_{12} deficiency was defined as plasma vitamin B_{12} concentrations less than 150 pmol/l.[19] Impaired vitamin B_{12} status was defined as plasma vitamin B_{12} < 150 pmol/l plus MMA > 0.26 μmol/l. cB_{12}, a combined indicator of vitamin B_{12} status, modified for three biomarkers (that is, vitamin B_{12}, MMA, tHcy or $3cB_{12}$), was calculated using the method and classification developed by Fedosov *et al*.[20] (that is, $cB_{12} = {}_{\log 10}[(\text{holoTC}*B_{12})/(\text{MMA}*\text{Hcy})]$—(age factor)). In Fedosov's method, the following cutoffs are used to categorize five levels of the combined indicator cB_{12}: probable deficiency ($cB_{12} < -2.5$), possible deficiency (−2.5 to < −1.5), low vitamin B_{12} (−1.5 to < −0.5), vitamin B_{12} adequacy (−0.5 to < 1.5) and elevated vitamin B_{12} ($cB_{12} \geqslant 1.5$).[20]

Variables were defined using conventional cutoffs, where available; otherwise, medians of variables were defined based on their distributions in this population. Non-normally distributed variables (that is, plasma vitamin B_{12}, MMA, tHcy, folate concentrations), were natural logarithmically transformed to ensure normality before analysis. Non-transformed values are presented in Tables 1 and 2, for interpretation purposes.

Linear and binomial regression models were used to examine the associations of maternal vitamin B_{12} biomarkers at each trimester with infant outcomes at 6 weeks of age, including vitamin B_{12} concentrations (continuous), vitamin B_{12} deficiency (categorical), methylmalonic acid (continuous) and homocysteine (continuous) concentrations.[21–23] Associations between maternal biomarkers of vitamin B_{12} status from each trimester and infant outcomes were examined independently in separate

Table 2. Maternal and infant vitamin B_{12} status

Variables	*Maternal*			*Infant*
	Trimester 1	*Trimester 2*	*Trimester 3*	*6 Weeks*
Gestational age at blood sample, weeks	10.57 (9.14, 12.57)	24.14 (23.71, 25.00)	33.14 (32.71, 33.57)	—
Plasma vitamin B_{12}, pmol/l	150 (103, 187)	151 (90, 220)	139 (88, 214)	155 (116, 229)
Vitamin B_{12} <150 pmol/l, *n* (%)	36 (50)	31 (50)	34 (59)	34 (44)
Plasma MMA, μmol/l	0.50 (0.31, 0.67)	0.35 (0.24, 0.57)	0.31 (0.16, 0.52)	0.12 (0.07, 0.24)
MMA >0.26 μmol/l, *n* (%)	58 (76)	45 (70)	30 (54)	15 (20)
Impaired vitamin B_{12} status,[a] *n* (%)	31 (43)	27 (44)	24 (43)	12 (16)
Plasma tHcy, μmol/l	9.06 (6.44, 12.91)	5.44 (3.38, 7.51)	5.47 (3.33, 8.22)	15.13 (10.39, 24.13)
cB12[b]	−0.80 (−1.28, −0.15)	−0.38 (−0.89, 0.16)	−0.19 (−1.04, 0.43)	—
Erythrocyte folate, nmol/l	399 (290, 487)	572 (420, 693)	508 (408, 638)	—

Abbreviations: IQR, interquartile range; MMA, methylmalonic acid; tHcy, total homocysteine. Values are median (IQR) and *n* (%). Statistical analyses: linear regression models were used to examine associations between maternal and infant vitamin B_{12} status; associations between maternal and infant biomarkers were significantly larger in the vitamin B_{12} intervention group, compared with the placebo group, using a linear regression model including vitamin B_{12} regimen, natural logarithmically transformed maternal biomarker and an interaction term ($P<0.05$). [a]Impaired vitamin B_{12} status: plasma vitamin $B_{12}<150$ pmol/l plus MMA >0.26 μmol/l. [b]cB12, a combined indicator of vitamin B_{12} status modified for three biomarkers (vitamin B_{12}, MMA and tHcy), was calculated using the method developed by Fedosov *et al*.[20]

models. Maternal vitamin B_{12} supplementation significantly increased maternal and infant plasma vitamin B_{12} concentrations in the aforementioned randomized trial;[15] therefore, vitamin B_{12} supplementation regimen was included as a covariate in all models. All models also included an adjustment for the gestational age at sample collection to account for variation in timing of samples. We used the Rothman and Greenland approach to evaluate an extensive list of potential confounders and identify covariates for inclusion in multivariate models, in which all known or suspected risk factors which led to >10% change in effect estimates were included in the model.[24] Additional baseline maternal risk factors for infant outcomes were included in multivariate models to evaluate the robustness of the observed associations, including the following: maternal education (⩾10th grade vs <10), standard of living index (⩾28 vs <28), total maternal lymphocyte counts, and maternal body mass index at baseline. The missing indicator method was used to retain observations with missing covariate data.[25] We also explored the potential interaction between the randomized intervention and biomarker outcomes, and potential effect modification of observed associations by the randomized intervention. Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The characteristics of participants included in this study are presented in Table 1. Baseline characteristics of pregnant women enrolled in the parent trial and current analysis were similar on age, socioeconomic status and nutritional indicators.

Vitamin B_{12} status during each trimester of pregnancy and in infants at 6 weeks of age ($n=77$) are presented in Table 2. At their first prenatal visit, ~51% of pregnant women had vitamin B_{12} deficiency (vitamin $B_{12}<150$ pmol/l), 43% had impaired vitamin B_{12} status (vitamin $B_{12}<150$ pmol/l plus MMA >0.26 μmol/l) and 38% had low folate status (erythrocyte folate <340 nmol/l). A total of 44% of infants were vitamin B_{12} deficient and 16% had impaired vitamin B_{12} status at 6 weeks of age.

The associations between maternal vitamin B_{12} status in each trimester and infant vitamin B_{12} concentrations at 6 weeks of age are presented in Table 3. Higher maternal plasma vitamin B_{12} levels in each trimester (T) were associated with higher vitamin B_{12} concentrations in infants' multivariate analyses, after adjusting for vitamin B_{12} supplementation status, gestational age of sample collection, maternal education, standard of living index, lymphocytes and body mass index. Similarly, vitamin B_{12} deficiency in each trimester was associated with lower vitamin B_{12} levels in infants in multivariate analyses, after adjusting for vitamin B_{12} supplementation and other socio-demographic factors. In contrast, higher maternal MMA concentrations predicted lower vitamin B_{12} concentrations in infants in multivariate analyses. After adjusting for vitamin B_{12} regimen, impaired maternal vitamin B_{12} status was associated with lower infant vitamin B_{12} levels. Higher maternal red blood cell folate levels were also associated with greater vitamin B_{12} concentrations in infants. In analyses that considered maternal vitamin B_{12} indicators alone or in combination, impaired maternal vitamin B_{12} status (that is, vitamin B_{12} deficiency and elevated MMA) was the strongest and most consistent predictor of infant vitamin B_{12} status. Findings were similar in both univariate and multivariate analyses, after adjusting for other variables.

The associations between maternal vitamin B_{12} status in each trimester of pregnancy and risk of vitamin B_{12} deficiency in infants at 6 weeks of age are presented in Table 4. Higher maternal vitamin B_{12} levels predicted lower risk of vitamin B_{12} deficiency in infants' multivariate analyses. Infants born to mothers who were vitamin B_{12}-deficient or who had impaired vitamin B_{12} status had a two to three times greater risk of vitamin B_{12} deficiency, after adjusting for the vitamin B_{12} regimen. In contrast, higher maternal MMA concentrations were associated with greater risk of infant vitamin B_{12} deficiency in multivariate analyses. Higher maternal folate levels were associated with lower risk of vitamin B_{12} deficiency in infants. Findings were similar in both univariate and multivariate analyses, after adjusting for potential confounders.

The associations between maternal vitamin B_{12} status in each trimester and infant MMA concentrations are presented in Table 5. Vitamin B_{12} deficiency and elevated MMA concentrations during pregnancy were associated with higher infant MMA concentrations. Impaired maternal vitamin B_{12} status during pregnancy also predicted higher MMA concentrations in infants. Higher folate levels during pregnancy were also associated with lower infant MMA concentrations.

The associations between vitamin B_{12} status in pregnancy and infant homocysteine levels are presented in Table 6. Higher vitamin B_{12} and folate levels during pregnancy predicted significantly lower infant tHcy concentrations. Maternal vitamin B_{12} deficiency and MMA levels were associated with significantly higher infant tHcy concentrations. Maternal impaired vitamin B_{12} status during pregnancy was also associated with higher tHcy concentrations in infants after adjusting for vitamin B_{12} regimen, although this was statistically significant in the second and third trimesters. There were no significant associations noted for maternal homocysteine and infant tHcy concentrations.

Table 3. Associations between maternal vitamin B_{12} status and infant vitamin B_{12} concentrations

Maternal Variables	*Trimester (T)*	*Model 1*[a]			*Model 2*[b]	
		n	*Beta (s.e.m.)*	P-*value*	*Beta (s.e.m.)*	P-*value*
Plasma vitamin B_{12},[c] pmol/l	T1	72	0.22 (0.12)	0.066	0.29 (0.13)	0.020
	T2	62	0.29 (0.11)	0.011	0.32 (0.11)	0.002
	T3	58	0.35 (0.10)	< 0.001	0.38 (0.10)	< 0.001
Vitamin B_{12} < 150 pmol/l	T1	72	− 0.29 (0.11)	0.012	− 0.38 (0.12)	0.001
	T2	62	− 0.29 (0.13)	0.027	− 0.32 (0.12)	0.009
	T3	58	− 0.47 (0.13)	< 0.001	− 0.50 (0.13)	< 0.001
Plasma MMA,[c] µmol/	T1	76	− 0.29 (0.08)	< 0.001	− 0.27 (0.08)	< 0.001
	T2	64	− 0.21 (0.09)	0.012	− 0.19 (0.09)	0.031
	T3	56	− 0.23 (0.08)	0.005	− 0.23 (0.08)	0.005
MMA > 0.26 µmol/l	T1	76	− 0.35 (0.13)	0.008	− 0.31 (0.13)	0.017
	T2	64	− 0.22 (0.14)	0.114	− 0.24 (0.13)	0.067
	T3	56	− 0.37 (0.13)	0.003	− 0.36 (0.13)	0.004
Impaired vitamin B_{12} status[d]	T1	72	− 0.41 (0.11)	< 0.001	− 0.45 (0.11)	< 0.001
	T2	62	− 0.19 (0.13)	0.160	− 0.25 (0.13)	0.048
	T3	56	− 0.47 (0.13)	< 0.001	− 0.50 (0.13)	< 0.001
Plasma tHcy,[c] µmol/l	T1	76	− 0.01 (0.09)	0.873	− 0.003 (0.09)	0.975
	T2	64	0.04 (0.11)	0.700	0.08 (0.11)	0.439
	T3	55	0.15 (0.10)	0.131	0.16 (0.10)	0.118
cB12[e]	T1	72	0.12 (0.06)	0.052	0.14 (0.06)	0.029
	T2	62	0.14 (0.07)	0.048	0.13 (0.06)	0.041
	T3	55	0.15 (0.07)	0.033	0.15 (0.07)	0.034
Erythrocyte folate,[c] nmol/l	T1	76	0.29 (0.16)	0.074	0.35 (0.15)	0.022
	T2	56	0.55 (0.19)	0.004	0.60 (0.18)	0.001
	T3	52	0.66 (0.23)	0.004	0.64 (0.23)	0.005

Abbreviations: MMA, methylmalonic acid; SLI, standard of living index; tHcy, total homocysteine. [a]Statistical analyses: linear regression models were used to examine associations between maternal vitamin B_{12} status and infant vitamin B_{12} concentrations; models were adjusted for vitamin B_{12} supplementation and gestational age of sample collection. [b]Statistical analyses: linear regression models were used to examine associations between maternal vitamin B_{12} status and infant vitamin B_{12} concentrations; models were adjusted for vitamin B_{12} supplementation, gestational age of sample collection, maternal education (⩾10th grade vs < 10), SLI (⩾28 vs < 28), baseline total lymphocyte count and baseline BMI. [c]Natural logarithmically transformed to achieve normality. [d]Impaired vitamin B_{12} status: plasma vitamin B_{12} < 150 pmol/l plus MMA > 0.26 µmol/l; [e]cB12, a combined indicator of vitamin B_{12} status modified for three biomarkers (vitamin B_{12}, MMA and tHcy), was calculated using the method developed by Fedosov *et al*.[20]

DISCUSSION

In this prospective analysis among pregnant women participating in a vitamin B_{12} supplementation trial, maternal vitamin B_{12} status during each trimester significantly predicted vitamin B_{12} status in infants at 6 weeks of age, even after adjusting for vitamin B_{12} supplementation. Infants born to mothers who were vitamin B_{12} deficient (< 150 pmol/l) or who had impaired vitamin B_{12} status (vitamin B_{12} < 150 pmol/l plus MMA > 0.26 µmol/l) had higher risk of being vitamin B_{12} deficient by 6 weeks of age, after adjusting for vitamin B_{12} regimen. Higher maternal vitamin B_{12} and folate status, but not maternal homocysteine, were associated with significantly lower infant tHcy concentrations. Impaired maternal vitamin B_{12} status, which combined both circulating and functional biomarkers, was the single best predictor of infant vitamin B_{12} status. Higher maternal folate concentrations in pregnancy were also associated with lower risk of vitamin B_{12} deficiency in infants.

The prevalence of vitamin B_{12} deficiency was high in this study as follows: 51% of mothers were vitamin B_{12} deficient and 42% had impaired vitamin B_{12} status at their first prenatal visit, and 44% of children had vitamin B_{12} deficiency at 6 weeks of age.[15]

Previous studies have noted correlations between maternal and neonatal vitamin B_{12} status at delivery.[18,26–28] For example, maternal and cord blood holoTC levels were significantly correlated at delivery in a cross-sectional study in Germany ($r = 0.68$, $P < 0.001$).[18] However, most research to date examining the associations between maternal and infant vitamin B_{12} status have been case–control or cross-sectional in design, and have relied on assessment of a single vitamin B_{12} biomarker at one time point (for example, maternal vitamin B_{12} concentrations at delivery), which constrains interpretation of findings.

Maternal vitamin B_{12} levels during pregnancy are thought to be associated with fetal[18,29] and infant[30] vitamin B_{12} concentrations. Some studies have noted significant associations between maternal and neonatal serum vitamin B_{12} concentrations, whereas prospective cohort studies in India[29] and Pakistan[31] have reported that neonatal vitamin B_{12} concentrations were 27% to twofold higher than maternal vitamin B_{12} concentrations. In the current study, infant vitamin B_{12} concentrations were not significantly different than maternal vitamin B_{12} concentrations in pregnancy. However, few studies to date have measured maternal vitamin B_{12} status prospectively throughout the course of pregnancy and examined its association with vitamin B_{12} status in their infants early in life.

This analysis included a comprehensive assessment of maternal vitamin B_{12} status prospectively throughout pregnancy, including both circulating (vitamin B_{12}) and functional (MMA, tHcy) vitamin B_{12} biomarkers. We also included impaired vitamin B_{12} status and calculated cB_{12} as a combined indicator of three vitamin B_{12} biomarkers (that is, vitamin B_{12}, MMA and tHcy), using methods developed by Fedosov *et al*.[20] In analyses that considered maternal vitamin B_{12} indicators alone or in combination impaired maternal vitamin B_{12} status (B_{12} < 150 pmol/l plus MMA > 0.26 µmol/l) was the strongest and most consistent predictor of infant vitamin B_{12} status. Vitamin B_{12} biomarkers were also assessed beginning early in gestation (⩽14 weeks), and maternal erythrocyte folate concentrations were assessed

Table 4. Associations between maternal vitamin B_{12} status and infant vitamin B_{12} deficiency

Maternal Variables	*Trimester (T)*	*Model 1*[a]			*Model 2*[b]	
		n	*RR (95% CI)*	P-*value*	*RR (95% CI)*	P-*value*
Plasma vitamin B_{12},[c] pmol/l (Ln)	T1	72	0.66 (0.41, 1.07)	0.094	0.41 (0.21, 0.78)	0.007
	T2	62	0.50 (0.30, 0.81)	0.005	0.41 (0.26, 0.65)	< 0.001
	T3	58	0.63 (0.42, 0.93)	0.019	0.46 (0.28, 0.74)	0.001
Vitamin B_{12} < 150 pmol/l	T1	72	1.93 (1.10, 3.38)	0.022	2.39 (1.42, 4.04)	0.001
	T2	62	2.69 (1.40, 5.16)	0.003	2.78 (1.48, 5.20)	0.001
	T3	58	3.01 (1.35, 6.73)	0.007	2.90 (1.41, 5.94)	0.004
Plasma MMA,[c] µmol/l	T1	76	2.51 (1.58, 3.99)	< 0.0001	2.60 (1.69, 4.01)	< 0.001
	T2	64	1.49 (0.95, 2.35)	0.085	1.73 (1.16, 2.58)	0.008
	T3	56	1.98 (1.16, 3.38)	0.013	2.08 (1.22, 3.56)	0.007
MMA > 0.26 µmol/l	T1	76	5.73 (1.39, 19.63)	0.014	4.97 (1.30, 18.97)	0.019
	T2	64	1.64 (0.80, 3.38)	0.180	1.71 (0.76, 3.84)	0.192
	T3	56	3.97 (1.75, 9.03)	0.001	3.81 (1.67, 8.67)	0.001
Impaired vitamin B_{12} status[d]	T1	72	2.53 (1.45, 4.42)	0.001	2.92 (1.74, 4.91)	< 0.001
	T2	62	1.82 (1.04, 3.19)	0.036	1.87 (1.03, 3.40)	0.040
	T3	56	3.49 (1.82, 6.66)	< 0.001	3.64 (1.92, 6.90)	< 0.001
Plasma tHcy,[c] µmol/l	T1	76	0.98 (0.69, 1.40)	0.914	0.95 (0.63, 1.45)	0.820
	T2	64	0.84 (0.54, 1.30)	0.426	0.76 (0.44, 1.30)	0.312
	T3	55	0.67 (0.45, 1.02)	0.059	0.74 (0.46, 1.17)	0.191
cB12[e]	T1	72	0.78 (0.57, 1.05)	0.100	0.68 (0.45, 1.02)	0.063
	T2	62	0.74 (0.54, 1.01)	0.057	0.65 (0.48, 0.88)	0.006
	T3	55	0.74 (0.56, 0.98)	0.035	0.68 (0.49, 0.95)	0.023
Erythrocyte folate,[c] nmol/l	T1	76	0.47 (0.25, 0.91)	0.025	0.46 (0.24, 0.86)	0.015
	T2	56	0.32 (0.15, 0.67)	0.003	0.26 (0.12, 0.58)	< 0.001
	T3	52	0.28 (0.10, 0.75)	0.012	0.31 (0.11, 0.86)	0.025

Abbreviations: BMI, body mass index; CI, confidence interval; MMA, methylmalonic acid; SLI, standard of living index; tHcy, total homocysteine. [a]Statistical analyses: binomial regression models were used to examine associations between maternal vitamin B_{12} status and infant vitamin B_{12} deficiency; models were adjusted for vitamin B_{12} supplementation and gestational age of sample collection. [b]Statistical analyses: binomial regression models were used to examine associations between maternal vitamin B_{12} status and infant vitamin B_{12} deficiency; models were adjusted for vitamin B_{12} supplementation, gestational age of sample collection, maternal education (⩾10th grade vs < 10), SLI (⩾ 28 vs < 28), baseline total lymphocyte count and baseline BMI. [c]Natural logarithmically transformed to achieve normality. [d]Impaired vitamin B_{12} status: plasma vitamin B_{12} < 150 pmol/l plus MMA > 0.26 µmol/l; vitamin B_{12} deficiency: vitamin B_{12} < 150 pmol/l. [e]cB12, a combined indicator of vitamin B_{12} status modified for three biomarkers (vitamin B_{12}, MMA and tHcy), was calculated using the method developed by Fedosov *et al*.[20]

Table 5. Associations between maternal vitamin B_{12} status and infant methylmalonic acid

Maternal variables	*Trimester (T)*	*Model 1*[a]			*Model 2*[b]	
		n	*Beta (s.e.m.)*	P-*value*	*Beta (s.e.m.)*	P-*value*
Plasma vitamin B_{12},[c] pmol/l	T1	76	−0.43 (0.23)	0.060	−0.41 (0.26)	0.113
	T2	63	−0.55 (0.20)	0.006	−0.52 (0.20)	0.009
	T3	58	−0.55 (0.20)	0.006	−0.49 (0.20)	0.016
Vitamin B_{12} < 150 pmol/l	T1	76	0.47 (0.23)	0.036	0.49 (0.25)	0.046
	T2	63	0.65 (0.23)	0.005	0.66 (0.23)	0.004
	T3	58	0.80 (0.26)	0.002	0.76 (0.26)	0.004
Plasma MMA,[c] µmol/	T1	76	0.46 (0.16)	0.003	0.47 (0.16)	0.003
	T2	63	0.60 (0.18)	0.001	0.64 (0.17)	< 0.001
	T3	58	0.36 (0.16)	0.022	0.32 (0.16)	0.049
MMA > 0.26 µmol/l	T1	76	0.66 (0.23)	0.005	0.63 (0.24)	0.008
	T2	63	0.56 (0.24)	0.020	0.66 (0.24)	0.006
	T3	58	0.46 (0.25)	0.062	0.42 (0.25)	0.084
Impaired vitamin B_{12} Status[d]	T1	76	0.54 (0.23)	0.018	0.51 (0.23)	0.029
	T2	63	0.63 (0.23)	0.006	0.68 (0.22)	0.002
	T3	58	0.72 (0.25)	0.004	0.68 (0.25)	0.007
Plasma tHcy,[c] µmol/l	T1	76	0.26 (0.15)	0.090	0.24 (0.16)	0.140
	T2	63	0.34 (0.18)	0.063	0.34 (0.20)	0.083
	T3	58	−0.03 (0.19)	0.869	−0.01 (0.19)	0.951
cB12[e]	T1	76	−0.36 (0.12)	0.003	−0.35 (0.12)	0.005
	T2	63	−0.35 (0.12)	0.003	−0.37 (0.13)	0.004
	T3	58	−0.29 (0.13)	0.030	−0.33 (0.14)	0.016
Erythrocyte folate,[c] nmol/l	T1	76	−0.87 (0.30)	0.004	−0.94 (0.29)	0.001
	T2	63	−0.65 (0.34)	0.058	−0.74 (0.35)	0.036
	T3	58	−1.58 (0.42)	0.0002	−1.50 (0.42)	< 0.001

Abbreviations: BMI, body mass index; MMA, methylmalonic acid; SLI, standard of living index; tHcy, total homocysteine. [a]Statistical analyses: linear regression models were used to examine associations between maternal vitamin B_{12} status and infant methylmalonic acid concentrations; models were adjusted for vitamin B_{12} supplementation and gestational age of sample collection. [b]Statistical analyses: linear regression models were used to examine associations between maternal vitamin B_{12} status and infant methylmalonic acid concentrations; models were adjusted for vitamin B_{12} supplementation, gestational age of sample collection, maternal education (⩾10th grade vs < 10), SLI (⩾ 28 vs < 28), baseline total lymphocyte count, baseline BMI. [c]Natural logarithmically transformed to achieve normality. [d]Impaired vitamin B_{12} status: plasma vitamin B_{12} < 150 pmol/l plus MMA > 0.26 µmol/l. [e]cB_{12}, a combined indicator of vitamin B_{12} status modified for three biomarkers (vitamin B_{12}, MMA and tHcy), was calculated using the method developed by Fedosov *et al*.[20]

Table 6. Associations between maternal vitamin B_{12} status and infant homocysteine

Maternal variables	*Trimester (T)*	*Model 1*[a]			*Model 2*[b]	
		n	*Beta (s.e.m.)*	P-*value*	*Beta (s.e.m.)*	P-*value*
Plasma vitamin B_{12},[c] pmol/l	T1	73	−0.001 (0.13)	0.993	−0.20 (0.14)	0.160
	T2	60	−0.27 (0.11)	0.017	−0.35 (0.11)	0.002
	T3	55	−0.39 (0.12)	0.016	−0.30 (0.11)	0.004
Vitamin B_{12} < 150 pmol/l	T1	73	0.02 (0.13)	0.845	0.18 (0.13)	0.161
	T2	60	0.28 (0.13)	0.035	0.33 (0.13)	0.010
	T3	55	0.42 (0.17)	0.013	0.38 (0.16)	0.017
Plasma MMA,[c] μmol/	T1	73	0.22 (0.09)	0.016	0.22 (0.09)	0.011
	T2	60	0.33 (0.11)	0.003	0.32 (0.10)	0.002
	T3	55	0.26 (0.10)	0.009	0.23 (0.09)	0.013
MMA > 0.26 μmol/l	T1	73	0.25 (0.14)	0.086	0.21 (0.14)	0.124
	T2	60	0.23 (0.14)	0.094	0.26 (0.13)	0.054
	T3	55	0.30 (0.15)	0.044	0.21 (0.14)	0.140
Impaired vitamin B_{12} status[d]	T1	73	0.06 (0.13)	0.627	0.18 (0.13)	0.155
	T2	60	0.39 (0.13)	0.002	0.42 (0.12)	0.001
	T3	55	0.44 (0.15)	0.004	0.41 (0.14)	0.004
Plasma tHcy,[c] μmol/l	T1	73	−0.09 (0.09)	0.297	−0.02 (0.09)	0.800
	T2	60	−0.02 (0.10)	0.865	0.06 (0.11)	0.549
	T3	55	−0.11 (0.11)	0.325	−0.05 (0.11)	0.631
cB12[e]	T1	73	−0.05 (0.07)	0.505	−0.11 (0.07)	0.108
	T2	60	−0.11 (0.07)	0.097	−0.15 (0.07)	0.028
	T3	55	−0.14 (0.08)	0.083	−0.15 (0.08)	0.048
Erythrocyte folate,[c] nmol/l	T1	73	−0.53 (0.16)	0.001	−0.56 (0.15)	< 0.001
	T2	60	−0.28 (0.19)	0.140	−0.36 (0.19)	0.051
	T3	55	−0.85 (0.24)	0.001	−0.73 (0.24)	0.002

Abbreviations: BMI, body mass index; MMA, methylmalonic acid; SLI, standard of living index; tHcy, total homocysteine. [a]Statistical analyses: linear regression models were used to examine associations between maternal vitamin B_{12} status and infant homocysteine concentrations; models were adjusted for vitamin B_{12} supplementation and gestational age of sample collection. [b]Statistical analyses: linear regression models were used to examine associations between maternal vitamin B_{12} status and infant homocysteine concentrations; models were adjusted for vitamin B_{12} supplementation, gestational age of sample collection, maternal education (⩾10th grade vs < 10), SLI (⩾28 vs < 28), baseline total lymphocyte count, baseline BMI. [c]Natural logarithmically transformed to achieve normality. [d]Impaired vitamin B_{12} status: plasma vitamin B_{12} < 150 pmol/l plus MMA > 0.26 μmol/l; [e]cB12, a combined indicator of vitamin B_{12} status modified for three biomarkers (vitamin B_{12}, MMA and tHcy), was calculated using the method developed by Fedosov *et al*.[20]

prospectively during pregnancy. In addition, the measurement of infant venous blood and comprehensive assessment of infant vitamin B_{12} status (that is, vitamin B_{12}, MMA and tHcy) were strengths of this analysis.

Our study had several limitations. The assessment of infant vitamin B_{12} status at a single time point (that is, at 6 weeks of age) and number of infant blood samples available for laboratory analyses ($n = 77$) limit interpretations of the associations between maternal vitamin B_{12} and infant status early in life. Our findings suggest that participants in the current study were similar to the parent-randomized trial on socio-demographic and nutritional variables; however, they may differ on other unmeasured covariates. Assessment of maternal vitamin B_{12} status beginning ⩽14 weeks gestation may not reflect periconceptional vitamin B_{12} status or the relevant etiologic period(s) for vitamin B_{12} status and perinatal outcomes. The cB_{12} measure developed by Fedosov *et al*.[20] and modifications for two, three or four biomarkers were constructed based on statistical models in non-pregnant (and primarily elderly) men and women in Chile, Denmark, United Kingdom, Ireland, and the United States. However, cB_{12} has not been investigated or validated in pregnant women or young infants, which represents a limitation and constrains the interpretation and generalizability of findings. In addition to total vitamin B_{12}, MMA and tHcy, assessment of maternal and infant holotranscobalamin may also represent a better circulating biomarker of vitamin B_{12} status and transportation from the maternal to the fetal circuit, as vitamin B_{12} enters and exits the placental villous tissue bound to transcobalamin.[32] Vitamin B_{12} metabolism is also influenced by other nutrients; assessment of infant folate status would further strengthen this analysis. Although findings in this study provide evidence of associations of maternal and infant vitamin B_{12} status within a randomized trial, the interpretation of these associations is not causal. Future research is needed to elucidate mechanisms of maternal–infant vitamin B_{12} transport, and the potential role of vitamin B_{12} in functional outcomes and child health.

In summary, in a large cohort of pregnant women participating in a randomized vitamin B_{12} supplementation trial in South India, vitamin B_{12} status throughout pregnancy significantly predicted vitamin B_{12} status in infants at 6 weeks of age, even after adjusting for vitamin B_{12} supplementation and several socio-demographic characteristics. Overall, impaired maternal vitamin B_{12} status, which combined both circulating and functional biomarkers, was the best predictor of infant vitamin B_{12} status. Infants who were born to women with vitamin B_{12} deficiency or those with impaired vitamin B_{12} status had two to four times greater risk of being vitamin B_{12} deficient, after adjusting for vitamin B_{12} supplementation status. Findings suggest that although prenatal vitamin B_{12} supplementation significantly improves vitamin B_{12} status, maternal vitamin B_{12} status early in pregnancy has an important role in determining vitamin B_{12} status early in life. Future research is needed to improve vitamin B_{12} status in women of reproductive age, and ensure optimal vitamin B_{12} status and health outcomes in pregnant women and their children.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: CD, KS, AVK and JLF designed the research; all the authors conducted the research; JLF conducted the data analysis and wrote the initial draft of the manuscript; and CD had the primary responsibility for the final content. All authors contributed to the interpretation of the data and in the development of this manuscript, and read and approved the final version.

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