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Vitamin B₁₂ intake and status in early pregnancy among urban South Indian women

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Abstract

Aim—To evaluate the vitamin B₁₂ status of South Indian women in early pregnancy and its relationship with sociodemographic, anthropometry and dietary intake.

Methods—Cross-sectional study among 366 pregnant urban South Indian women 14 weeks of gestation with outcome variables defined as low vitamin B₁₂ blood concentration (<150 pmol/L) and impaired vitamin B₁₂ status [low vitamin B₁₂ plus elevated methylmalonic acid (MMA) >0.26 µmol/L].

Results—Low plasma vitamin B₁₂ concentration was observed in 51.1% of the women, while 42.4% had impaired B₁₂ status. Elevated MMA, elevated homocysteine (>10 µmol/L) and low erythrocyte folate (<283 nmol/L) was observed among 75.8%, 43.3% and 22.2% of women, respectively. The median (25th, 75th percentile) dietary intake of vitamin B₁₂ was 1.25 (0.86, 1.96) µg/day. Lower maternal body weight was associated with higher vitamin B₁₂ concentration [prevalence ratios (PR) (95% CI) 0.57 (0.39, 0.84)]. The predictors of impaired vitamin B₁₂ status were non-use of yoghurt [PR (95% CI) 1.63 (1.03, 2.58)], non-use of fish [PR (95% CI) 1.32 (1.01, 1.71)] and primiparity [PR (95% CI) 1.41 (1.05, 1.90)].

Conclusion—A high prevalence of vitamin B₁₂ deficiency in early pregnancy among urban South Indian women was related to primiparity and to a low consumption of yoghurt and fish.

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The authors' responsibilities were as follows:

TMS was involved in data collection, data analyses and wrote the first draft of the manuscript. CD was involved in conception of study design, supervision of data collection and contributed to manuscript writing. TT was involved in data analysis and contributed to writing of the manuscript. RB was involved in data analysis and contributed to manuscript writing. RR was involved in data collection. SMV contributed to manuscript writing. KS was involved in conception of study design, supervision of data collection and contributed to manuscript writing. AVK was involved in the conception of the study design and contributed to writing of manuscript.

Keywords

Pregnancy; urban South Indian women; plasma vitamin B₁₂; Methylmalonic acid; homocysteine; food frequency questionnaire; dietary vitamin B₁₂ intake

Introduction

Deficiency of vitamin B₁₂ is considered to be highly prevalent in India and the metabolic signs of vitamin B₁₂ deficiency have been reported in 75% of adult men and women from urban areas of West India [1]. Pregnancy is a critical stage during the life cycle when the requirement for vitamin B₁₂ increases due to the rapid cell multiplication resulting from the enlargement of the uterus, placental development and fetal growth [2]. Vitamin B₁₂ deficiency during pregnancy may elevate plasma Hcy levels [3], and is associated with an increased risk for adverse outcomes including neural tube defects [4, 5], small-for-gestational-age [6], intrauterine growth retardation [7], early miscarriage [8, 9] and pre-eclampsia [1].

In addition to increased requirements during pregnancy, chronic low intakes of dietary vitamin B₁₂ [10] and/or malabsorption due to parasitic infections and other causes [11] may lead to a negative vitamin B₁₂ balance and depletion of tissue stores leading to a deficiency state. Pregnant women who are vegetarian or those who consume low amounts of animal products are more likely to become vitamin B₁₂ deficient, and to give birth to infants who develop clinical or biochemical signs of B₁₂ deficiency and to have low levels of this vitamin in their breast milk [12]. The vitamin B₁₂ status of an infant at birth as well as stores during infancy is strongly determined by the amount of vitamin B₁₂ that is accumulated by the fetus during pregnancy [13]. Assessing the vitamin B₁₂ intake and status of pregnant women consuming diets low in foods from animal sources may allow the identification of those with sub-optimal status and at risk for adverse pregnancy and birth outcomes. In addition, early pregnancy may be a better time to assess the vitamin B₁₂ status since later in pregnancy there may be reductions in the plasma concentrations of vitamin B₁₂ and its metabolites due to plasma volume expansion [14].

We therefore conducted a cross sectional study among 366 pregnant urban South Indian women 14 weeks of gestation to assess vitamin B₁₂ status based on blood concentrations of plasma B₁₂, methylmalonic acid (MMA) and Hcy. In addition, we evaluated the relationship between demographic, anthropometric and dietary intake patterns with biochemical assessment of B₁₂ status.

Subjects and methods

Study design and study population

A cross-sectional study was performed among a cohort of pregnant women enrolled in a randomized controlled trial of vitamin B₁₂ supplementation (NCT00641862). The study was conducted at Hosahalli Referral Hospital, Bangalore which is a government maternity health care center predominantly catering to the needs of the women from the lower socioeconomic strata of urban Bangalore. Pregnant women were enrolled in early pregnancy (14 weeks

gestation) from December 2008 to November 2010. 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 subject at enrolment.

Inclusion and exclusion criteria

Pregnant women aged 18 and 40 years, 14 weeks of gestation and registered for antenatal screening at the hospital were included in the study. Women who were diagnosed with chronic illness such as diabetes mellitus, hypertension, heart disease or thyroid disease, those tested positive for HbSAg, HIV or syphilis, those who were likely to move out of the city prior to delivery, those with multiple gestation, those treated for infertility, those with previous caesarean section or those who were already consuming vitamin B₁₂ supplements were excluded from the study.

Recruitment and lost to follow up

We contacted 1376 women at the antenatal clinic during the study period. Of these 958 women were excluded for the following reasons: 836 women planned to deliver outside Bangalore at their maternal home town; 67 women wanted to terminate the pregnancy; seven women had a history of hypertension; four women were < 18 years; four women had a previous caesarean section and pregnancy was not confirmed among 40 women. There were 418 women who were eligible to participate in the study. Of these, 52 declined to participate, i.e. 366 consented.

Sociodemographic and anthropometric information

Sociodemographic information was obtained by trained research assistants through interview. Gestational age (in weeks) was calculated from the reported first day of the last menstrual period (LMP). Weights of all the mothers were recorded using a digital balance (Salter's 9016, Tonbridge, Kent, UK) to the nearest 100 g, while height was measured using a stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kg divided by the square of height in meters (kg/m²).

Dietary data

A pre-tested interviewer-administered FFQ was used to assess the habitual dietary intake for the 3 months preceding the date of the subject'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 [15] and has a food list of 127 items, derived from a food database developed from studies at St John's Medical College. To develop this database, the raw food items required for each recipe were entered and the nutrient and food group values were obtained for the cooked weight of that recipe. Cooked weight of the recipes was obtained by a recipe collection and standardization process. Databases of recipes were obtained from urban and rural groups. The food items were cooked in the metabolic kitchen according to the recipes provided and weights were obtained for the edible portion of the food used in the recipes. The Indian Food Composition tables were used to estimate the nutrient content of the raw ingredients reported in the

recipes [16]. Nutrient content of the cooked recipe was obtained by using a conversion factor accounting for the weight/volume changes in cooking [15]. Nutrient and food group values were computed by multiplying the frequency of intake by the portion size and the nutrient or food group value of each food item. These were then summed up to obtain the nutrient and food group values for all foods consumed per day. Total amount of the food groups consumed per day was also calculated (in grams). The food groups were cereals (whole grains, processed grains), lentils, green leafy vegetables, roots and tubers, fruits, milk and milk products, red meat, organ meat, poultry, fish, nuts, coffee and tea. For further analyses, the milk and milk products group was subdivided into milk, yoghurt and other dairy products.

Biochemical data

Approximately 10 ml of blood was drawn from subjects after an overnight fast by venipuncture and collected in both ethylene diaminetetraacetate (EDTA) and plain vacutainers (BD Franklin Lakes, New Jersey, USA). Hemoglobin (Hb) and complete blood count were analysed on whole blood samples in an automated Coulter counter (ABX Pentra C+, Horiba medicals, CA, US). The plasma and red blood cells were separated and stored at -80°C until analysis for vitamin B₁₂, Hcy, MMA and erythrocyte folate.

Vitamin B₁₂ was measured by the electrochemiluminescence method (Elecsys 2010, Roche Diagnostics Mannheim, USA), while the combined measurement of Hcy and MMA was performed by gas chromatography-mass spectrometry (GC-MS) method (Varian 3800, Palo Alto, CA, USA) [17]. The intra- and inter-day assay coefficients of variation (CV) for vitamin B₁₂ were 0.54 and 2.44 respectively. The inter-day assay CV for MMA and Hcy was 5.57 and 5.04 respectively while the intra-day assay CV was 6.92 and 5.60 respectively. Erythrocyte folate was measured by a competitive immunoassay with direct chemiluminescence detection on an automated immunoanalyser (ADVIA Centaurs, Bayer Health Care Diagnostics, Tarrytown, New York) [18]. The folate concentration in the hemolysate was converted to values for whole blood by adjusting for the hematocrit.

A single stool sample was collected from the pregnant women and analyzed immediately for the presence of helminthic ova, cysts and trophozoites by the wet mount method [19]. We primarily tested the stool samples for the presence or absence of *Giardia lamblia* and *Enterobius Vermicularis*.

Statistical analysis

Continuous data were summarised as means (SD) and categorical data as numbers (%). Data that are normally distributed are expressed as mean (SD). Skewed variables such as dietary intake variables, biochemical variables, household income and gestational age at recruitment have been reported as median (25th, 75th percentile). Anemia was defined as Hb < 11.0 g/dL [20]. In the absence of validated cut-offs for plasma vitamin B₁₂, MMA, and Hcy concentrations in pregnant Indian women, we used the cut-offs available from the literature. Low vitamin B₁₂ concentration was defined as plasma vitamin B₁₂ concentration < 150 pmol/L [1]. Elevated MMA was defined as > 0.26 $\mu\text{mol/L}$ [1]. Elevated Hcy was defined as > 10.0 $\mu\text{mol/L}$. Low erythrocyte folate concentration was defined as < 283 nmol/L [21].

Since vitamin B₁₂ deficiency may exist with normal plasma vitamin B₁₂ concentration and since plasma vitamin B₁₂ may not reliably indicate vitamin B₁₂ status [22], and the use of MMA is a relatively specific indicator of vitamin B₁₂ deficiency [23], we created a composite variable termed as impaired vitamin B₁₂ status (low B₁₂ concentration and elevated MMA level) to identify women with confirmed B₁₂ deficiency. Secondary analyses included evaluating predictors of women with low plasma vitamin B₁₂ concentration alone, as well as predictors of women with impaired vitamin B₁₂ status.

The association of impaired vitamin B₁₂ status with maternal sociodemographic and anthropometric characteristics and intake of specific foods were examined using multivariable log-binomial regression analyses. For the analysis to identify predictors of low vitamin B₁₂ concentration the log binomial model did not converge and Poisson regression was applied. The food groups that were consumed by less than 50% of the respondents were considered as binary (consumed/not consumed) and the others were considered as three categories (not consumed, below median consumption and above median consumption). All sociodemographic, anthropometric and dietary variables were examined with low B₁₂ concentrations and impaired B₁₂ status. The variables that were significant in this analysis with $P < 0.20$ were considered for log binomial regression analyses or the Poisson regression. The characteristics found significant in the univariate analysis with $P < 0.20$ were considered for the multivariable analysis. Prevalence ratios (PR) with 95% confidence intervals and corresponding P values for both unadjusted and adjusted models are presented. Variables were retained in the final adjusted regression model if they had a $P < 0.05$. Statistical analyses were carried out with SPSS (version 16, SPSS, Chicago, IL., USA). Log-binomial regression analysis was carried out using the PROC GENMOD program in SAS software (version 9.2 SAS, Cary, N.C., USA).

Results

The sociodemographic, anthropometric, dietary intake and biochemical data are presented in Table 1. The mean (SD) age of the pregnant women was 22.6 (3.7) years and the mean (SD) gestational age at the time of the study was 11.2 (2.4) weeks. Primiparous women made up 64.5% of the cohort. The median dietary intake of energy, protein, and folate among pregnant women was lower in comparison to the recommended dietary allowances (RDA) for this group [24]. The median (25th, 75th percentile) dietary intake of vitamin B₁₂ was 1.25 (0.86, 1.96) µg/day. None of the women used iron or folate or vitamin B₁₂ supplements on the study entry.

The mean (SD) Hb was 11.5 (1.5) g/dL and the prevalence of anemia (Hb < 11.0 g/dL) was 30.3% (111/366). Mean corpuscular volume was greater than 90 fL in 10.1% of the women. A very small percentage of pregnant women (0.5%) had macrocytic anemia (anemia with mean corpuscular volume > 90 fL). About half (51.1%) of the women had low plasma vitamin B₁₂ concentrations (< 150 pmol/L). MMA levels > 0.26 µmol/l was observed in three-fourth (75.8%) of the pregnant women. About half (43.3%) of the pregnant women had Hcy levels > 10.0 µmol/L. Nearly a quarter (22.2%) of the pregnant women in our cohort had erythrocyte folate concentrations < 283 nmol/L. Impaired vitamin B₁₂ status as

evidenced by plasma B₁₂ < 150 pmol/L and MMA > 0.26 µmol/L was observed among 42.4% of the women. Giardia lamblia cysts were present in 3.0% of the women.

In the women in the cohort, energy-adjusted dietary vitamin B₁₂ intake correlated significantly with plasma B₁₂ concentration ($r = 0.164$, $P = 0.002$), but not with plasma MMA ($P = 0.260$) or Hcy ($P = 0.902$) level. The dietary intake of folate did not correlate either with the erythrocyte folate ($P = 0.276$) or with Hcy ($P = 0.532$). Figure 1a shows the association between plasma vitamin B₁₂ concentration and MMA level. There was a significant inverse correlation between plasma vitamin B₁₂ concentration and plasma MMA ($r = -0.184$, $P = 0.001$). Figure 1b shows the association between plasma vitamin B₁₂ concentration and level of Hcy. There was an inverse correlation between plasma vitamin B₁₂ concentration and plasma Hcy, which, however, was of borderline statistical significance ($r = -0.097$, $P = 0.069$).

Table 2 shows the results of Poisson regression analyses of low vitamin B₁₂ concentration as well as log-binomial regression analyses of impaired vitamin B₁₂ status (defined by plasma B₁₂ concentration < 150 picomol/L plus MMA > 0.26 µmol/L) with sociodemographic and anthropometric factors. In the univariate analyses, pregnant women in the lowest third of body weight had the lowest risk for low vitamin B₁₂ concentration [PR (95% CI): 0.59 (0.46, 0.78)]. Maternal age, gestational age in weeks, parity, income and education were not significantly associated with low vitamin B₁₂ concentrations in the univariate analyses. When we considered impaired vitamin B₁₂ status as the outcome variable, we observed that primiparous women had a significantly higher risk of impaired vitamin B₁₂ status in comparison to multiparous women [PR (95% CI): 1.55 (1.16, 2.08)]. Maternal age, gestational age in weeks, maternal weight, income and education were not significantly associated with impaired vitamin B₁₂ status in the univariate analyses.

In the multivariable model after adjusting for the other variables in the model, lower maternal body weight was associated with lower risk for low vitamin B₁₂ concentration [PR (95% CI): 0.57 (0.39, 0.84)]. The risk for impaired vitamin B₁₂ status was higher among primiparous women [PR (95% CI): 1.41 (1.05, 1.90)].

Table 3 shows the results of Poisson regression analyses of low vitamin B₁₂ concentration as well as log-binomial regression analyses of impaired vitamin B₁₂ status with nutritional factors. In the univariate analyses intake of milk and milk products, red meat and poultry, eggs, organ meat, and fish were not associated with low vitamin B₁₂ concentration. Intake of milk and milk products was associated with the higher prevalence of impaired vitamin B₁₂ status [PR (95% CI): 3.04 (1.17, 7.90)]. Since we were interested to know the specific milk or milk product that was associated with vitamin B₁₂ deficiency, we categorized them as milk, yoghurt and other dairy products (cottage cheese, buttermilk and payassam). Pregnant women who did not consume yoghurt had a higher risk for impaired B₁₂ status in comparison to those who consumed it above the median intake [PR (95% CI): 1.64 (1.03, 2.61)].

In the multivariable model, women reporting no intake of yoghurt had a higher risk for impaired vitamin B₁₂ status [PR (95% CI): 1.63 (1.03, 2.58)]. No reported dietary intake of

fish was also associated with a greater risk for impaired vitamin B₁₂ status in the multivariable analyses [PR (95% CI): 1.32 (1.01, 1.71)].

Discussion

In a cohort of pregnant women from urban South India recruited early in pregnancy, we found a high prevalence of biochemical vitamin B₁₂ deficiency. Low plasma vitamin B₁₂ concentration and impaired vitamin B₁₂ status was observed in 51.1% and 42.4% of the pregnant women, respectively. Pregnant women in our cohort had a higher prevalence of low plasma vitamin B₁₂ concentration and elevated MMA levels in comparison to pregnant women from Nepal [25] as well as from other developed countries when assessed in early pregnancy [26, 27, 28].

Studies from both developed and developing countries have documented a high prevalence of low vitamin B₁₂ concentration as well as high level of MMA among pregnant women in the 2nd and 3rd trimester of pregnancy [29, 30]. Low plasma vitamin B₁₂ concentration has also been reported among pregnant women from both rural and urban India in the later half of pregnancy [31, 32, 33]. However, concentration of vitamin B₁₂ and vitamin B₁₂ dependent metabolites such as Hcy and MMA are known to decline during the course of pregnancy [34, 35, 36, 37], mainly due to factors such as hemodilution and hormonal influences in addition to nutritional deficiencies [2]. Therefore low values of vitamin B₁₂ and its metabolites later in pregnancy must be interpreted with caution. Our study partially overcomes this issue of declining B₁₂ concentration by recruiting women as early as 14 weeks of gestation. Although maternal plasma volume begins to increase by 6 weeks of gestation, the peak increase in plasma volume is observed only between 30 to 34 weeks of gestation [14]. Pregnant women in our cohort also had a higher prevalence of folate deficiency (22.2%) in early pregnancy in contrast to pregnant women from rural Pune for whom a very low prevalence of folate deficiency (0.2%) was reported even in the second and third trimester of pregnancy [38]. It may be that the pregnant women in the present study had an overall poor dietary intake that was reflected not just in their low consumption of foods from animal sources but also low intake of green leafy vegetables. Access to and consumption of fresh vegetables may also be limited in an urban compared to rural setting.

Inadequate dietary intake is one of the main causes of vitamin B₁₂ deficiency. In our cohort, dietary intake of vitamin B₁₂ was significantly related to plasma B₁₂ concentration, although the correlation was weak. Other studies in postmenopausal women as well as young and elderly men and women have reported a much stronger correlation between dietary vitamin B₁₂ intake and plasma or serum B₁₂ levels [39, 40]. However, the vitamin B₁₂ intake among these men and women were much higher than those reported among pregnant women from our cohort, and were contributed by vitamin B₁₂ supplements in addition to diet. The women in our cohort not only had a low dietary intake of vitamin B₁₂ but also were not consuming either B₁₂ supplements or foods fortified with vitamin B₁₂ at the time of recruitment. The weak correlation observed between diet and plasma vitamin B₁₂ concentration may also be due to non-diet sources contributing to B₁₂ intake among pregnant women in our cohort. For example, it has been speculated that small intestinal microbes such as *Pseudomonas* and *Klebsiella* may synthesize nutritionally significant amounts of vitamin B₁₂ for terminal ileal

absorption [41]. In addition, since the food tables we used were based on raw foods, micronutrient losses during cooking and food preparation may be one of the causes of poor correlation between calculated dietary intake and plasma B₁₂. Since data on cooking duration was not available in this sample, applying a uniform correction factor for cooking losses could only adjust for the systematic error and did not alter the observed association between diet and plasma B₁₂. With a dietary intake of 1.25 µg/day of vitamin B₁₂, and based on the assumption that 50% of the dietary B₁₂ is absorbed by healthy adults with normal gastric function [42], the amount of absorbed vitamin B₁₂ would only be 0.63 µg/day. More recently, the Indian Council of Medical Research has defined an intake of 1.2 µg/day of vitamin B₁₂ to be adequate to meet the requirements of all pregnant women in India since Indians consume a predominantly vegetarian diet [24, 43]. This is in contrast to the FAO/WHO recommendation of 2.6 µg/day for pregnant women [44], where requirements are based on intakes of a population subsisting mainly on animal foods.

From a food group perspective, the intake of foods from animal sources was not associated with low vitamin B₁₂ concentrations. However, intake of fish was associated with a lower risk for impaired vitamin B₁₂ status. Dairy products are important sources of vitamin B₁₂, and the absorption of vitamin B₁₂ from them is considered to be more efficient in comparison to that from poultry, fish or meat sources [42]. However, we did not observe any significant relation between intake of milk and low vitamin B₁₂ concentration or impaired B₁₂ status, in contrast to the observations among adult and elderly men and women from Norway where the dietary intake of milk was a significant contributor to vitamin B₁₂ status [45]. It may be that milk intake among our pregnant women was not as high as the milk intake reported among adult and elderly men and women from Norway, which ranged between 173 to 320 ml/day. Equally, milk in Norway is primarily consumed in the raw form (mild pasteurization) and along with meals, unlike in India where milk is boiled prior to consumption. Boiling of milk for 5 to 30 minutes leads to 30 – 50% loss in the vitamin B₁₂ content [46].

More interestingly, we found that yoghurt intake was a significant predictor of B₁₂ status. An intake of 100 ml of yoghurt would provide approximately the same amount of B₁₂ as a similar portion of milk (0.357 µg), however there is a possibility that certain *Lactobacilli* may synthesize B-vitamins during fermentation of milk. For example, particular strains of *Leuconostoc* and *Propionibacterium* have been shown to increase the vitamin B₁₂ content substantially during fermentation of milk [47]. In Egyptian children with elevated Hcy levels, yoghurt containing *Lactobacillus acidophilus* administered over a period of 42 days was effective in increasing B₁₂ producing bacteria in the gut, increasing plasma levels of vitamin B₁₂, folate and reducing Hcy and urinary MMA [48]. In addition to a poor diet, parasitic infestations may result in sub-optimal vitamin B₁₂ status. For instance, infection with *G. lamblia* is known to result in vitamin B₁₂ malabsorption in children [49]. *G. lamblia* infections were also significant predictors of low holotranscobalamin II concentrations among lactating women from Guatemalan at three months postpartum [50]. However, among pregnant women in our cohort, the prevalence of *G. lamblia* infections were very low (3%) and was not significantly associated with low B₁₂ concentration. Equally interesting was the observation that among pregnant women in our cohort, higher body weight was

significantly associated with impaired B₁₂ status. The relation between maternal body weight and impaired B₁₂ status may be bidirectional. Vitamin B₁₂ acts as a coenzyme for methylmalonylCoA mutase that converts methylmalonyl CoA to succinyl CoA. The accumulation of MMA in a vitamin B₁₂ deficient person can affect mitochondrial respiration [51, 52], and therefore impair substrate oxidation. Equally, it may be possible that overweight or obesity may lead to changes in the absorption, excretion or metabolism of vitamin B₁₂, and this may be exacerbated by a diet low in vitamin B₁₂ [53].

The strength of our study is that it is the first of its kind from India that investigates the biochemical indicators of vitamin B₁₂ status including MMA and Hcy, in addition to plasma B₁₂ concentration among pregnant women early in pregnancy. In addition, we used a validated FFQ with a trained interviewer with portion sizes to minimize recall bias. However, it may be difficult to generalize our study findings to all Indian pregnant women since our cohort was an urban, health facility-based one. In addition, the generalizability of our findings may be affected by the high exclusion rate in our cohort due to mothers planning to deliver outside Bangalore.

In conclusion, we found a high prevalence of vitamin B₁₂ deficiency among urban south Indian pregnant women 14 weeks of gestation. The association of higher maternal body weight with low vitamin B₁₂ concentration merits further evaluation. Efforts towards improving the vitamin B₁₂ status of pregnant women should focus on improving the overall quality of the diet and on increasing the intake of specific foods that are high in vitamin B₁₂ and commonly consumed in a vegetarian population. In addition, it remains to be explored with well-designed intervention studies, whether supplementing vulnerable groups such as pregnant women with vitamin B₁₂ in addition to iron and folate would improve haematological and clinical outcomes during pregnancy.

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Figure 1a.

Association between plasma vitamin B₁₂ concentration and MMA level among South Indian pregnant women.

Plasma vitamin B₁₂ concentration versus concentration of methylmalonic acid (MMA) in pregnant South Indian women ($r = -0.184$, $P = 0.001$, $n = 349$). The dotted line on the x axis denotes the cut off level for vitamin B₁₂ deficiency (< 150 picomol/L), while the dotted line on the y axis denotes the cut off level for elevated MMA (> 0.26 μmol/L).

Figure 1b.

Association between plasma vitamin B₁₂ concentration and Hcy level among South Indian pregnant women.

Plasma vitamin B₁₂ concentration versus concentration of homocysteine (Hcy) in pregnant South Indian women ($r = -0.097$, $P = 0.069$, $n = 349$). The dotted line on the x axis denotes the cut off level for vitamin B₁₂ deficiency (< 150 picomol/L), while the dotted line on the y axis denotes the cut off level for elevated Hcy (> 10 μmol/L).

Sociodemographic, anthropometric, dietary and biochemical characteristics of pregnant south Indian women (n = 366).

Table 1

<i>Parameter</i>	
<i>Sociodemographic characteristics</i>	
<i>Age (years)(n= 366) ¹</i>	22.6 ± 3.7
<i>Level of Education (n=364) ²</i>	
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%)
<i>Occupation (n=364) ²</i>	
Unemployed	305 (83.8%)
Unskilled Worker	21 (5.8%)
Skilled Worker	27 (7.4%)
Others (Secretarial jobs, teachers, shop owners) business, shop owner	11 (3.1%)
Total monthly household income(INR)(n=365) ³	6000 (4500, 9000)
<i>Parity (n=366) ²</i>	
0	236 (64.5%)
1	130 (35.6%)
Gestational age at recruitment by LMP (weeks)(n=366) ¹	11.2 ± 2.4
<i>Anthropometric characteristics (n=364) ¹</i>	
Weight (kg)	47.8 ± 8.1
Height (cm)	153.0 ± 5.6
Body mass index (kg/m ²)	20.4 ± 3.3
<i>Dietary intake characteristics (n=362) ³</i>	
Energy (Kcal/day)	1695 (1447, 1977)
Protein (g/day)	51.1 (42.4, 58.9)
Fat (g/day)	46.2 (38.3, 55.1)
Carbohydrates (g/day)	269 (225, 315)

Parameter	
Vitamin B ₁₂ (µg/day)	1.25 (0.86, 1.96)
Folate (µg/day)	272 (226, 318)
Intake of B ₁₂ or folate supplements	Nil
Biochemical characteristics	
Hemoglobin (g/dL) (n = 366) ¹	11.5 ± 1.5
Anemia (Hb < 11.0 g/dL) (n = 366) ²	111 (30.3%)
Macrocytosis (MCV > 90)	37 (10.1%)
Anemia and MCV > 90 ²	2 (0.5%)
Plasma B ₁₂ level (pmol/L) (n = 352) ³	149.3 (109.4, 204.5)
Methylmalonic acid level (µmol/L) (n = 360) ³	0.47 (0.28, 0.67)
Homocysteine level (µmol/L) (n = 360) ³	9.22 (5.74, 15.08)
Erythrocyte folate (nmol/L) (n = 359) ³	386.9 (290.6, 496.4)
Plasma B ₁₂ < 150 pmol/L (n = 352) ²	179 (51.1%)
Prevalence of elevated MMA levels (MMA > 0.26 µmol/L) (n = 360) ²	273 (75.8%)
Prevalence of elevated homocysteine levels (Hcy > 10.0 µmol/L) (n = 360) ²	157 (43.3%)
Prevalence of impaired vitamin B ₁₂ status (plasma B ₁₂ < 150 pmol/L & MMA>0.26 µmol/L) (n = 349) ²	148 (42.4%)
Prevalence of low erythrocyte folate levels (erythrocyte folate < 283 nmol/L) (n = 351) ²	80 (22.2%)
Prevalence of Giardia lamblia infections (n = 328) ²	10 (3.0%)

LMP, Last menstrual period, MCV: Mean Corpuscular Volume

¹ Mean ± SD

² Numbers (%)

³ Median (25th, 75th percentile)

Sociodemographic and anthropometric factors associated with low vitamin B₁₂ concentration and impaired vitamin B₁₂ status among pregnant Indian women.

Table 2

Parameter	Low vitamin B ₁₂ concentration ¹ (Plasma vitamin B ₁₂ < 150 picomol/L)			Impaired vitamin B ₁₂ status ² (Plasma vitamin B ₁₂ < 150 picomol/L and MMA > 0.26 µmol/L)		
	% low plasma B ₁₂	Univariate PR 95% CI	P value	Adjusted PR ³ 95% CI	P value	% impaired B ₁₂ status
<i>Sociodemographic and anthropometric characteristics</i>						
Maternal age (years)			0.732			0.791
Lowest third (20-90) (n = 122)	48.3	1.02 (0.77, 1.36)	0.889			43.0
Middle third (21.0-23.40) (n = 125)	53.7	1.03 (0.80, 1.33)	0.819			44.7
Highest third (< 23.40) (n = 119)	50.9	1.00				40.2
Gestational age in weeks			0.053		0.144	0.415
Lowest third (9.60) (n = 123)	41.7	1.00		1.00		38.9
Middle third (9.70-12.60) (n = 130)	55.6	1.33 (1.02, 1.74)	0.036	1.37 (0.94, 1.99)	0.101	47.2
Highest third (>12.60) (n = 113)	55.5	1.77 (1.02, 3.05)	0.042	1.35 (0.92, 1.99)	0.126	41.4
Maternal weight (kg)			0.003		0.024	0.064
Lowest third (43.30) (n = 123)	38.5	0.59 (0.46, 0.78)	0.001	0.57 (0.39, 0.84)	0.004	34.5
Middle third (43.40-51.0) (n = 121)	49.6	0.59 (0.37, 0.93)	0.022	0.79 (0.55, 1.12)	0.186	43.6
Highest third (>51.0) (n = 121)	64.7	1.00		1.00		49.6
Parity						1.00
Primiparous (n=227)	54.6	1.23 (0.98, 1.55)	0.076	1.24 (0.89, 1.73)	0.335	48.9
Multiparous (n=124)	44.4	1.00		1.00		31.5
Income (INR)			0.330		0.655	0.165
Lowest third (n=152)	48.0	1.00		1.00		38.0

Parameter	Low vitamin B ₁₂ concentration ¹ (Plasma vitamin B ₁₂ < 150 picomol/L)				Impaired vitamin B ₁₂ status ² (Plasma vitamin B ₁₂ < 150 picomol/L and MMA > 0.26 µmol/L)			
	% low plasma B ₁₂	Univariate PR 95% CI	P value	Adjusted PR ³ 95% CI	P value	% impaired B ₁₂ status	Univariate PR 95% CI	P value
Middle third (n=93)	49.5	1.03 (0.94, 1.50)	0.827	0.96 (0.66, 1.40)	0.830	42.6	1.12 (0.82, 1.53)	0.476
Highest third (n=105)	57.1	1.19 (0.95, 1.69)	0.146	1.17 (0.82, 1.66)	0.390	50.0	1.32 (0.99, 1.74)	0.055
Education			0.411					0.267
Illiterate/Primary (n=90)	50.0	0.86 (0.64, 1.17)	0.335			40.0	0.81 (0.56, 1.17)	0.271
High School (n=170)	50.0	1.02 (0.80, 1.31)	0.849			50.0	1.05 (0.79, 1.40)	0.739
> High School (n=87)	50.0	1.00				40.0	1.00	

Table 3

Nutritional factors associated with low vitamin B₁₂ concentration and impaired vitamin B₁₂ status among pregnant Indian women.

Parameter	Low vitamin B ₁₂ concentration ⁵ (Plasma vitamin B ₁₂ < 150 picomol/L)			Impaired vitamin B ₁₂ status ⁶ (Plasma vitamin B ₁₂ < 150 picomol/L and MMA > 0.26 µmol/L)		
	% low plasma B ₁₂	Univariate PR 95% CI	P value	Adjusted PR ³ 95% CI	P value	Adjusted PR ⁴ 95% CI
Intake of foods						
Poultry and meat (g/day)			0.182		0.419	0.422
= 0.0g/day (n = 74)	60.6	1.28 (0.99, 1.65)	0.063	1.28 (0.84, 1.96)	0.258	1.16 (0.85, 1.58)
0.1 – 17.30 g/day (n = 146)	49.6	1.05 (0.82, 1.33)	0.717	1.04 (0.73, 1.46)	0.865	0.94 (0.71, 1.24)
> 17.30 g/day (n = 146)	47.5	1.00	1.00	1.00	42.4	1.00
Eggs (g/day)			0.616			0.899
= 0.0g/day (n = 112)	53.8	1.13 (0.88, 1.46)	0.347		43.8	1.07 (0.79, 1.45)
0.1 – 10.17 g/day (n = 125)	52.1	1.09 (0.85, 1.41)	0.479		43.2	1.05 (0.78, 1.42)
> 10.17 g/day (n = 125)	47.5	1.00	1.00		41.0	1.00
Organ meat consumption						
No (n = 330)	52.2	1.35 (0.86, 2.13)	0.150	1.36 (0.73, 1.46)	0.330	1.35 (0.80, 2.29)
Yes (n = 32)	38.7	1.00	1.00	1.00	32.3	1.00
Fish consumption						
No (n = 234)	54.2	1.20 (0.96, 1.51)	0.104	1.19 (0.85, 1.67)	0.314	1.24 (0.94, 1.63)
Yes (n = 128)	45.1	1.00	1.00	1.00	36.9	1.00
Milk (ml/day)			0.284			0.313
= 0.0 ml/day (n = 175)	55.4	1.21 (0.86, 1.72)	0.269		45.8	1.37 (0.88, 2.14)
0.1 – 120.0 ml/day (n = 144)	47.5	1.04 (0.73, 1.49)	0.831		42.0	1.26 (0.79, 1.99)
> 120.0 ml/day (n = 47)	45.7	1.00	1.00		33.3	1.00

Parameter	Low vitamin B ₁₂ concentration ⁵ (Plasma vitamin B ₁₂ < 150 picomol/L)				Impaired vitamin B ₁₂ status ⁶ (Plasma vitamin B ₁₂ < 150 picomol/L and MMA > 0.26 µmol/L)					
	% low plasma B ₁₂	Univariate PR 95% CI	P value	Adjusted PR ³ 95% CI	P value	% impaired B ₁₂ status	Univariate PR 95% CI	P value	Adjusted PR ⁴ 95% CI	P value
Yoghurt (ml/day)			0.031		0.263			0.021		0.035
= 0.0 ml/day (n = 171)	51.8	1.45 (0.99, 2.12)	0.056	1.58 (0.96, 2.61)	0.071	43.9	1.64 (1.03, 2.61)	0.038	1.63 (1.03, 2.58)	0.038
0.1 – 27.8 ml/day (n = 135)	56.5	1.58 (1.08, 2.32)	0.019	1.60 (0.97, 2.64)	0.066	48.1	1.79 (1.23, 2.87)	0.015	1.70 (1.07, 2.72)	0.025
> 27.8 ml/day (n = 60)	35.7	1.00		1.00		26.8	1.00		1.00	
Other dairy products										
No (n = 69)	51.5	1.01 (0.78, 1.31)	0.926			47.0	1.13 (0.84, 1.51)	0.423		
Yes (n = 297)	50.9	1.00				41.7	1.00			

PR, Prevalence ratio, All values are PR and 95% CI

P- Values (two-sided) have been reported for the adjusted PR.

¹ Multivariable Poisson regression of low vitamin B₁₂ concentration, maternal age, maternal weight, gestational age at recruitment, education, income, parity,

² Multivariable log-binomial regression of impaired vitamin B₁₂ status, maternal age, maternal weight, gestational age at recruitment, education, income, parity.

³ Adjusted PR from a Poisson regression model adjusted for the effects of the other variables in the model.

⁴ Adjusted PR from a log-binomial regression model adjusted for the effects of the other variables in the model.

⁵ Multivariable Poisson regression of low vitamin B₁₂ concentration, intake of poultry and meat, eggs, organ meat, fish, milk, yoghurt and other dairy products.

⁶ Multivariable log-binomial regression of impaired vitamin B₁₂ status, intake of poultry and meat, eggs, organ meat, fish, milk, yoghurt and other dairy products.