Original Article

Vitamin B_{12} and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B_{12} and high folate status

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Maternal vitamin B_{12} deficiency and hyperhomocysteinemia predict poor pregnancy outcome, foetal adiposity and insulin resistance. In India amongst practicing clinicians and policy makers there is little appreciation of widespread vitamin B_{12} deficiency. We investigated 163 (86 rural, 77 urban) pregnant women attending antenatal clinics in a rural health centre and a referral hospital in the city of Pune, at 17, 28, and 34 weeks gestation for vitamin supplements, and circulating concentrations of vitamin B_{12}, folate, and total homocysteine. At enrolment 80% rural and 65% urban women had low vitamin B_{12} but only two rural women had low folate concentrations. During pregnancy 85% rural and 95% of urban women received folic acid; 12% rural and 84% urban women also received vitamin B_{12}. In women receiving no supplementation (n=17) plasma vitamin B_{12} and folate did not change from 17 to 34 weeks gestation, but homocysteine increased (p<0.05). Homocysteine concentrations at 34 weeks gestation in women receiving only folic acid (n=71, mean 8.4 (95% CI 7.8, 9.1) µmol/L) were comparable to the unsupplemented group (9.7 (7.3, 12.7), p=0.15), but women who received a total dose of > 1000 µg of vitamin B_{12} up to 34 weeks (n=42, all with folic acid) had lower concentrations (6.7 (6.0, 7.4), p<0.001). Increasing dose of vitamin B_{12} (r_s=-0.31, p=0.006) but not folic acid (r_s=-0.19, p=0.11) was associated with lower plasma total homocysteine concentration. In vitamin B_{12} insufficient, folate replete pregnant women, vitamin B_{12} supplementation is associated with a reduction of plasma total homocysteine concentration in late pregnancy.

Key Words: vitamin B_{12}, folate, homocysteine, pregnancy, Indian

INTRODUCTION

Vitamin B_{12} and folate play a vital role in one carbon (1-C) metabolism which is crucial for the general wellbeing of pregnancy and particularly for fetal growth. Circulating total homocysteine (tHcy) concentrations are a useful integral marker of 1-C metabolism, and are influenced by genetic and dietary factors including intake of vitamins B_{12}, folate, pyridoxine and riboflavin. Maternal hyperhomocysteinemia is associated with adverse pregnancy outcomes including early abortions,[1] neural tube defects (NTD),[2] preterm labour and intrauterine growth retardation.[3,4] In the predominantly non-vegetarian Western population, hyperhomocysteinemia is usually attributed to folate deficiency. On the other hand, in the predominantly vegetarian Indians who eat only small amounts of animal derived foods, hyperhomocysteinemia is usually associated with vitamin B_{12} deficiency.[5,6] In the Pune Maternal Nutrition Study, two thirds of pregnant mothers had low levels of circulating vitamin B_{12} concentrations but only an occasional mother had low folate concentration; a third had high tHcy concentrations but 90% had high circulating methylmalonic acid concentrations which is specific indicator of vitamin B_{12} deficiency.[4] Maternal hyperhomocysteinemia was associated with intrauterine growth retardation,[4] and maternal low vitamin B_{12} concentrations with adiposity, insulin resistance and poor neuro-cognitive performance during childhood.[5,7] A study from Bangalore also reported that maternal vitamin B_{12} deficiency predicted intrauterine growth retardation.[8] These results suggest that maternal 1-C metabolism and vitamin B_{12} as well as folate nutrition could have a crucial role not only in the high prevalence of intrauterine growth retardation, but also in the current epidemic of diabetes and cardiovascular disease in India. This is a novel explanation for Developmental Origins of Health and Disease theory in India.[9]

There is little appreciation of the widespread vitamin B_{12} deficiency amongst the practicing clinicians and policy

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Manuscript received 21 April 2009. Initial review completed 6 October 2009. Revision accepted 18 March 2010.

makers in India. There are no national guidelines to help obstetricians to prescribe proper doses of vitamin B_{12} and folic acid to pregnant women. The majority of obstetricians read western guidelines which are based on folic acid supplementation trials to prevent NTD in B_{12} adequate non vegetarian European women. The National Anaemia Prophylaxis Programme in India mandates folic acid and iron (0.5 mg and 60 mg, respectively) supplementation to young girls and pregnant women, and obstetricians prescribe large doses of folic acid to prevent NTD although the majority of pregnant women present to the antenatal clinic more than 28 days after conception when the neural tube has closed.[10,11] Therefore there is an urgent need to study current practices of vitamin supplementation of pregnant Indian women and the relationship between supplement dose and circulating vitamin B_{12}, folate and tHcy concentrations.

We studied vitamin supplement intake in apparently healthy rural and urban pregnant women in Pune and investigated its relationship with circulating vitamin B_{12}, folate and tHcy concentrations.

MATERIALS AND METHODS

In this observational, non-interventional study, we aimed to involve approximately 100 pregnant women from rural farming community and 100 from a lower middle-class urban community (housewives and office workers). The rural women attended the antenatal clinic at King Edward Memorial (KEM) Hospital's outreach programme at Vadu Rural Health Centre and the urban women attended the antenatal clinic at KEM Hospital, Pune. Vadu Rural Health Centre is situated approximately 50 km from Pune city and serves many surrounding villages; approximately 350 women deliver in this centre every year. King Edward Memorial Hospital is a 550-bed teaching hospital that mostly caters for the lower middle-class population in Pune city. It provides primary as well as referral antenatal services to women from surrounding areas and an estimated 1500 women deliver their infants there every year.

We screened women attending the antenatal clinics between May 2004 and February 2006. Gestation was calculated from the last menstrual period and verified by ultrasonography. If there was a difference of more than two weeks, ultrasonographic gestation was used. Those beyond 21 weeks gestation, with a multiple or an abnormal pregnancy, with adverse obstetric history (previous caesarean section, intrauterine growth retardation, foetal death, neonatal death, pre-eclampsia, twin pregnancy) or a chronic medical condition (diabetes, hypertension, infective illness etc) were excluded. Women who agreed to take part, signed an informed consent, and the study was approved by the KEM Hospital Research Centre's ethics committee.

We enrolled 184 (94 rural, 90 urban) eligible women and arranged for them to have an overnight stay (visit) at the Diabetes Unit, KEM Hospital Research Centre three times during pregnancy. The mean (range) of gestation at the three visits were 17 (12-22), 28 (24-30), and 34 (32-36) weeks) (Figure 1).

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (CMS Instruments, London, UK) and body weight was recorded in fasting state to the nearest 0.005 kg (Conveigh, Electronic Inst. P. Ltd., Mumbai, India).

At each visit a research officer (obstetrician) interviewed the women in detail, for medical history, treatment, and supplementation. These women were treated by different obstetricians, though in the same institute. It was not intended to influence their treatment but only to study current practices and their effect on circulating tHcy, vitamin B_{12} and folate concentrations. The prescriptions were studied to extract the following information; the start date, number of tablets, vitamin B_{12} and folic acid content per tablet and the number of days for which they were consumed. If women were taking any other supplements, they were also recorded. Total supplemented dose of B_{12} and folic acid received up to the last study visit

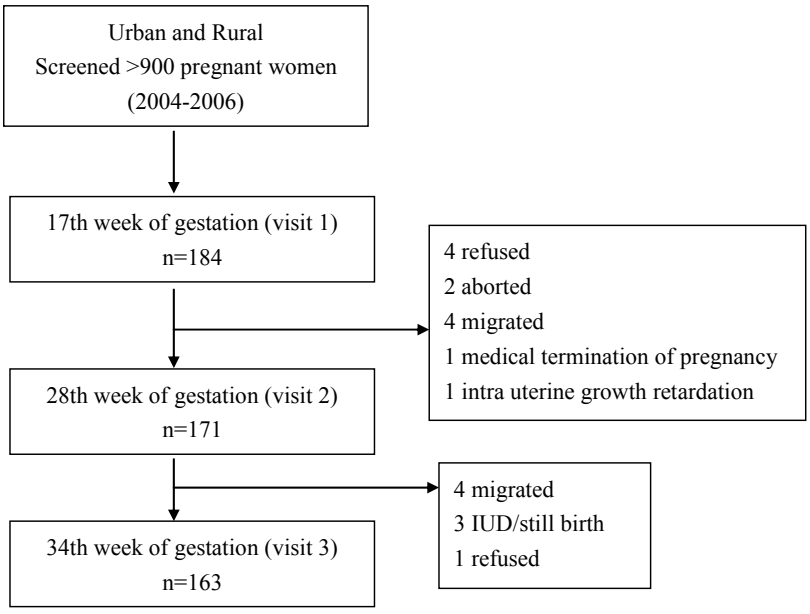

Figure 1. Flow chart to demonstrate inclusion in final analysis of 163 pregnant women at 3 stages of pregnancy.

(34^{th} week) was calculated from the information provided at each visit. Three groups were formed according to supplementation; those receiving no supplementation, those receiving only folic acid, and those receiving B_{12} with folic acid. One woman, receiving vitamin B_{12} injection, was excluded for analysis on the association between supplements and biochemical outcomes.

A trained nutritionist administered a food frequency questionnaire at each visit to assess the frequency of consuming foods rich in vitamin B_{12} and folate. We identified the following foods as relatively high sources of vitamin B_{12}: non-vegetarian foods (egg, fish, chicken and meat), and milk and milk products (yoghurt, curd and cheese). Green leafy vegetables (spinach, coriander, fenugreek, colocassia and other locally available green leafy vegetables) were identified as the main source of folate. For each food item, frequency of consumption in pregnancy and the normal portion size were ascertained and the quantity of B_{12} and folate consumed were estimated from established Indian values.[12]

At all three visits fasting blood samples from the antecubital vein were collected in the sitting position, in an EDTA vacutainer. Haemoglobin was measured on a Beckman Coulter Analyzer ($A^{C\cdot}T$ diff ^{TM}Analyzer, Miami, Florida, USA). The remaining blood was centrifuged (4°C, 2500 *g* x 15 min) within an hour of collection, and plasma was stored at -70ºC until further analysis. Plasma cobalamin was measured by microbiological assay (MBA) using a colistin sulfate-resistant strain of *Lactobacillus leichmanii*.[13,14] Total plasma folate was measured by MBA using a chloramphenicol-resistant strain of *Lactobacillus casei*.[15,16] Plasma tHcy was measured by fluorescence polarization immunoassay, using Axsym (Abbott, IL, USA).[17] In our laboratory, for vitamin B_{12}, folate, and tHcy analysis, between-day coefficients of variations were < 8%, <7% and <3% respectively.

Statistical analysis

Data are presented as mean (SD), median (25-75 centile) and percentages dependent on the distribution of the data. Plasma concentrations of vitamin B_{12} and tHcy were log transformed to meet the requirement for parametric tests and back transformed, mean and 95% confidence intervals are reported. Associations were investigated with Spearman's rank correlation coefficient (r_s). Kruskal-Wallis and Mann-Whitey U tests were used for non parametric comparisons. Vitamin B_{12} deficiency was defined as <150 pmol/L, folate deficiency as <7 nmol/L, hyperhomocysteinaemia as >10 µmol/L and anaemia as haemoglobin <110 g/L.[18,19] Vitamin supplement intake was categorised as "nil" (no folic acid, no vitamin B_{12}), folic acid only, and 3 groups of increasing supplemented doses of vitamin B_{12} (≤1000 µg, 1001 to 2000 µg, and >2001µg, during the total study period). Repeated measures ANOVA was used to investigate biochemical changes in the non-supplemented group at the three measurements points with post-hoc LSD tests of difference. ANOVA with linear trend analyses was performed to determine the effect of dose on biochemical outcomes for the difference between baseline and 34 weeks, by supplementation groups i.e. "nil" (n=17), only folic acid (n=71) and increasing total supplemented dose of B_{12} (n=32, 27, 15 for the three groups of B_{12} dose). We also adjusted for baseline values (17 week). Post hoc Tukey honestly significant difference was used to examine for homogenous subsets with respect to folic acid dose received. We calculated the amount of vitamin consumed per day, from the total intake and the number of days. Analyses were performed using SPSS 16.0 for windows (SPSS, Chicago, IL, USA).

RESULTS

Out of 184 women, 21 women who did not complete all three visits were not different (data not shown) with respect to physical characteristics and circulating vitamin B_{12}, folate and tHcy concentrations compared to 163 women who completed all three gestational visits. The characteristics of these women (86 rural and 77 urban) at enrolment (17 weeks) are shown by location in Table 1. Rural women were younger, shorter and lighter than urban, they were also less educated and had lower socioeconomic status. At enrolment, 56% rural and 57% urban women were anaemic; and at 34 weeks, 62% rural and 53% urban women were anaemic. One woman had macrocytosis (red cell mean corpuscular volume of >100 fL) at the second and third visit (data not shown). The pattern

Table1. Characteristics of the women at enrolment (values are controlled for gestation)

	Total (n=163)	Rural (n=86)	Urban (n=77)	*p*-value
Age (year)	22.8±3.7	21.4±2.8	24.3±3.9	<0.001
Height (cm)	153.9±5.7	152.9±5.3	155.1±6.0	0.015
Weight (kg)	48.5±7.0	46.2±5.3	51.0±7.8	<0.001
BMI (kg/m^2)	20.5±2.9	19.7±2.1	21.3±3.5	0.001
Education (year)	10.8±3.5	9.5±3.1	12.0±3.5	<0.001
Haemoglobin g/L	107±13	107±13	106±16	0.780
<110 (g/L) %	56	56	57	0.860
Plasma vitamin B_{12} (pmol/L)	119 (87, 161)	112 (83, 140)	127 (95, 169)	0.015
<150 (pmol/L) %	73	80	64	0.020
Plasma folate (nmol/L)	33.1±19.3	28.8±18.5	37.9±19.2	0.002
<7 (nmol/L) %	1	2	0	0.340
Plasma tHcy (µmol/L)	8.3 (6.5, 10.1)	8.6 (6.7, 10.2)	8.5 (6.0, 10.3)	0.150
>10 (µmol/L) %	27	28	26	0.780

Data are shown as mean ±SD, median (interquartile range) or prevalence (%), p unpaired Student's *t* test or Mann-Whitney U for non-normal data, and Chi squared test for prevalence.

of vitamin supplementation by location is shown in Table 2. Only 9/85 (10.6%) rural women received B_{12} with their supplement compared to 65/77 (84.4%) of urban women.

From the semi-quantitative food frequency questionnaire, 44 (27%) women never ate any non-vegetarian foods (egg, fish, chicken and meat) and only 18 (11%) ate non-vegetarian foods at least every second day. Milk consumption was also low: eleven (7%) never consumed and 45 (28%) consumed milk "less than every second day"; the average portion size of milk was 150 ml (approximately 0.21 µg B_{12}). We estimated that the average daily dietary intake of vitamin B_{12} did not exceed 0.4 µg even if milk and non-vegetarian foods were consumed. Among those with B_{12} supplements, the average vitamin B_{12} intake from supplements was approximately 8µg/day. Therefore we did not adjust for dietary intake in the analysis.

Rural women had lower plasma vitamin B_{12} and folate concentrations but tHcy concentrations were not different than urban (Table 1). Prevalence of vitamin B_{12} deficiency was higher in rural than in urban (80% vs. 64%, p=0.02); only 2 rural but none of the urban women were folate deficient throughout pregnancy.

Green leafy vegetables, fruit and pulses are good sources of folate and were consumed frequently by rural and urban women. At least every alternate day green leafy vegetables and pulses were consumed by 90% and 60% respectively; and 60% of the women consumed fruits every day. It is estimated that about 50% of dietary folate is destroyed by Indian cooking. Taking this into account, we estimated that the average intake of folate at all 3 points in pregnancy was in the order of 0.14 mg/d. Given much higher content of folic acid in the supplements (see below) we did not adjust for dietary folate in the analysis.

Folic acid content of the supplements varied from 0.25 mg to 5 mg per tablet. In early pregnancy 5 mg tablets were commonly used. Over the whole period 89% of women received folic acid at any stage; 75% at the first visit, 64% at the second and 66% at the third. None of the women received folic acid before conception and only four had received before 28 days of gestation. About 50% of the women taking folic acid supplements received >1mg/d; the upper limit for daily dose of folic acid. The vitamin B_{12} content of the supplements varied from 0.2 µg to 100 µg. Over the whole period 46% women received B_{12} at any visit; 29% of women received vitamin B_{12} at the first visit, 28% at second and 28% at third visit. None of the women had received vitamin B_{12} before conception. The most frequent supplemented dose of vitamin B_{12} was 15µg/d.

Dates of when supplementation started and ended, as well as the doses consumed were variable therefore the cumulative dose of supplementation up to the last visit is reported. Seventeen (13 rural, 4 urban) women received no supplements throughout pregnancy, 71 women received folic acid only while 74 received folic acid with vitamin B_{12}.

The median total folic acid dose up to 34 week in the folic acid only group was 126 (range 23 to 750) mg [995 (range 192 to 7653) µg/day] which was similar to the median dose in those who received folic acid with B_{12}, median 142 (range 90 to 210) mg (p=0.39, Mann-Whitney U). The median total B_{12} supplement dose received was 1125 (range 455 to 1950) µg. Significantly more urban women received folic acid than rural (95% vs 85%, p<0.033); and 84% urban vs 12% rural (p<0.001) also received B_{12}.

We have used the non-supplemented group as a "natural" control to study serial changes in vitamin $B_{12,}$ folate and tHcy concentrations during pregnancy (Table 3). With no supplementation, haemoglobin and plasma folate concentrations fell between 17 and 28 weeks gestation but increased at 34 weeks to a concentration similar to week 17. Plasma vitamin B_{12} concentrations remained similar at the three visits but tHcy concentration was significantly higher at 34 weeks gestation (p=0.02).

Figure 2, summarises vitamin $B_{12,}$ folate, tHcy and haemoglobin concentrations in different supplemented groups. At 17 weeks plasma tHcy concentrations were

Table 2. Prevalence of dosage regime by location and within total sample (n=162)

Group	Rural n (% within location)	Urban n (% within location)	Total
No supplementation	13 (15.3%)	4 (5.2%)	17 (10.5%)
Only folate	63 (74.1%)	8 (11.3%)	71 (43.8%)
Folate +<1000 µg B_{12}	7 (8.1%)	25 (32.5%)	32 (19.8%)
Folate +1000 to<2000 µg B_{12}	0	27 (35.1%)	27 (16.7%)
Folate +>2000 µg B_{12}	2 (2.4%)	13 (16.9%)	15 (9.3%)

Supplementation regime was reported at gestation weeks 17, 28 and 34 and total dose consumed up to week 34 calculated.

Table 3. Serial circulating concentrations of vitamin B_{12}, folate, tHcy and haemoglobin in unsupplemented pregnant women (n=17).

Gestation	Vitamin B_{12} (pmol/L)	Plasma folate (nmol/L)	tHcy (µmol/L)	Haemoglobin (g/L)
17 weeks	116 (86, 154)	25.2 (17.5, 32.8)	8.8 (6.7, 11.5)	105(99, 112)
28 weeks	112 (93, 134)	16.7 (11.4, 22.1)*	9.2 (7.2, 11.8)	96 (89, 102)*
34 weeks	110 (89, 137)	22.7 (13.7, 31.7)‡	9.7 (7.3, 12.7)	103 (95, 111)‡

Mean (95%CI for mean); * refers to significant difference between week 17 and 28 week and ‡ between week 28 and 34 (p<0.05).

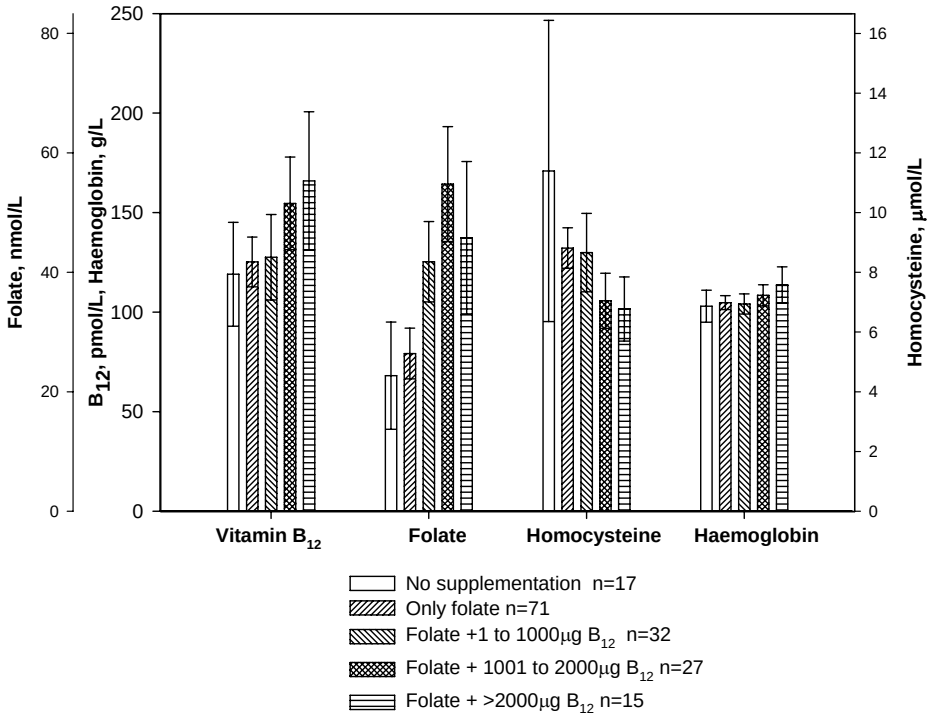

Figure 2. Mean concentrations (95% CI) of plasma B_{12}, folate, tHcy and haemoglobin concentrations at gestation week 34 by categories of vitamin supplementation. The first category had no supplementation, the second received folic acid only, and the third, B_{12} supplementation (with folic acid) groups are divided into three progressively increasing doses. In each analysis, the linear trend for increase or decrease (ANOVA linear unweighted term) is significant.

similar in all five groups, overall mean 8.2 (95% CI 7.8, 8.7) µmol/L p=0.47, ANOVA for difference between groups (data not shown). At 34 weeks, there was no significant difference in the change from 17 weeks to 34 weeks in circulating concentrations of haemoglobin, vitamin B_{12}, folate and tHcy in those who received no supplementation and those who received folic acid only. In particular, the increase in tHcy was not different (1.1 (95% CI 0.04, 2.1) µmol/L vs. 0.1 (95% CI -0.4, 0.6) µmol/L respectively, p=0.60). Compared to those who received no supplementation or folic acid only women who received up to 1000 µg vitamin B_{12} also had a non significant decrease in plasma tHcy concentration -0.6 (95% CI -1.4, 0.4) µmol/L . In contrast, women who received a total dose of supplemented vitamin B_{12} of more than 1000 µg (groups combined, n= 42, all with folic acid) plasma tHcy concentrations decreased by 1.3 (95% CI 0.4, 2.2) µmol/L which was significantly different to the unsupplemented group (p<0.006), even after adjustment for baseline value (p=0.052). In the group receiving B_{12} with folic acid there was a negative association of plasma tHcy concentration at 34 weeks with total supplemented dose of vitamin B_{12} (r_s -0.31, p=0.006) but not with total supplemented dose of folic acid (r_s=-0.19, p=0.11). Haemoglobin showed a linear trend that increased with vitamin B_{12} dose category, p=0.02 (Figure 2). Among the B_{12} subgroups, the total folic acid dose did not differ (p=0.607 for linear trend from ANOVA).

DISCUSSION

In this prospective, observational study we have shown in a group of rural and urban pregnant Indian women that prescription of folic acid and vitamin B_{12} varied widely. Our results shows that the plasma tHcy concentration at 34 weeks gestation was lower in those who received vitamin B_{12} supplementation compared to those who received only folic acid or no supplementation. Increasing supplemented dose of B_{12} was associated with progressively lower plasma tHcy concentration. There was no difference in tHcy concentration between unsupplemented and only folic acid supplemented group.

The study provides interesting insights into current practices of prescription of folic acid and vitamin B_{12} in an Indian urban teaching hospital and a nearby rural primary health care centre attached to this hospital. First, none of the women took vitamin B_{12} supplementation before the neural tube would have closed. Second, the dose of folic acid prescribed was variable. The majority received more than 1 mg per day, i.e. higher than safe upper limit in the western world, and 60% women received 5 mg or more per day, at least for sometime during pregnancy. Third, a substantial number of women continued to receive folic acid into late pregnancy, presumably to prevent anaemia and also preeclampsia. However, folic acid alone did not seem to have any effect on haemoglobin concentrations. An evaluation of the National Anaemia Prophylaxis Programme failed to show a reduction in the incidence of anaemia in pregnancy.[10] Fourth, supplementation was more common in the urban than in the rural practice which reflects the differences in the standards of clinical care as well as the socio-economic and educational characteristics of the women. The difference was substantial for vitamin B_{12} partly reflecting the awareness of the hospital doctors of the research in our unit. Finally, none of the women received folic acid and B_{12} supplements before conception.

In unsupplemented European women, plasma B_{12} and tHcy concentrations progressively decrease during preg-

nancy.[20-23] This contrasts to the findings in our unsupplemented women where there was no significant change in plasma vitamin $B_{12,}$ but tHcy concentrations increased between 17 and 34 weeks of gestation. Folate (and haemoglobin) fell between 17 and 28 weeks and then increased back to original levels at week 34. Progressive reductions of folate concentrations during the course of pregnancy have been reported by others.[21,22,24,25] Explanations include haemodilution,[26,27] accelerated utilisation of folate because of participation in increased cellular biosynthesis and active transport across the placenta.[28-31] While plasma folate concentration was in normal range in almost all women at all measurement points, in the 17 unsupplemented women, plasma B_{12} concentration was below 150 pmol/L in 15 and tHcy was more than 10 µmol/L in 7 at 34 weeks gestation.

In vegetarian and elderly vitamin B_{12} deficient subjects, plasma tHcy concentrations are higher than normal despite high normal concentrations of serum folate.[32-34] Unless both micronutrients are bio-available, the accumulation of tHcy cannot be prevented.[35,36] This is also reflected in our results which show that plasma tHcy concentrations at 34 weeks in the only folic acid supplemented group were comparable to those in the unsupplemented group, while they were lower in those who received vitamin B_{12} supplementation. It is likely that a part of high folate concentrations is contributed by "trapped" folate due to deficiency of vitamin B_{12}, which prevents methyl-tetrahydrofolate from being recycled again into the pool of active folates.[37] Vitamin B_{12} releases "trapped" folate allowing homocysteine to be remethylated to methionine, resulting in a reduction of plasma tHcy concentrations. Dietary intake of B_{12} by these women was much lower than that in the prescribed supplements. Plasma tHcy concentration was similar in women who were unsupplemented, supplemented with only folic acid and those supplemented with a total of <1000 µg of vitamin B_{12}. It was significantly lower in those who received total supplemention of >1000 µg of vitamin $B_{12.}$ In this study there was no association between dietary intake measures and circulating tHcy, B_{12} and folate; unlike the Pune Maternal Nutrition Study,[5] where frequency of consumption of non-vegetarian foods and milk was predictive of plasma B_{12} and tHcy concentration. This could be attributed to the effect of supplementation in this study, and also possibly to smaller numbers. Cobalamin deficiency during pregnancy in the presence of high normal folate has also been reported in Nepalese and European women with vegetarian diets, so the situation described in this paper is not just applicable to Indians.[34,38]

Our findings have some limitations but they have to be viewed in light of absence of comparable data from India. It was not easy to ascertain the true supplementation dose because we could not verify women's compliance; however, an experienced obstetrician recorded a detailed history of every woman at each visit. Admittedly, our study represents observations in one teaching hospital and its attached rural primary health centre. However, in the absence of national guidelines and any relevant data these findings are of importance to practicing obstetricians as well as to policy makers. Because the neural tube closes by 28 days of gestation and the majority of pregnant Indian women first attend an antenatal clinic much later,[11] folic acid supplementation by obstetricians following Western guidelines to prevent neural tube defect miss the critical periconceptual period. It is also not appreciated that the Western recommendation is based on clinical trials in predominantly non-vegetarian eating populations. The MRC vitamin study research trial used 4 mg folic acid for preventing recurrent neural tube defect while the Hungarian trial used 0.8 mg (along with 4 µg vitamin B_{12} and other vitamins) for prevention of a first occurrence of neural tube defect.[39,40] The standard recommendation is to use 0.4mg daily peri-conceptionally. The Indian Council of Medical Research trial was for the prevention of *recurrent* NTDs and was prematurely terminated when the results of the MRC Vitamin Study Research Group were published.[39,41] It showed a nonsignificant 58% reduction in incidence of recurrent NTDs. The commonly available folic acid tablet in India is 5 mg. The majority of obstetricians have an impression that the water soluble folic acid is harmless in large doses, and are unaware of the current concerns about high dose folic acid fortification and supplementation,[42] especially in vitamin B_{12} deficient populations. Thus, we feel that education of care-givers as well as population (especially young girls and their families) will be important in improving the situation. This is crucial because India has high rates of NTD (ranging from 3 to 11 per 1000 pregnancies).[43] Recently, there is a suggestion for universal fortification with folic acid of flour and other products, but vitamin B_{12} is not considered.[44] The prescription of B_{12} supplements in the urban mothers in our study possibly reflects the influence of our previous research while the much lower rate in rural practice suggests a need for spreading the research findings more widely. It is of note that even in the urban hospital, vitamin B_{12} was not prescribed for all women.

In summary, in B_{12} insufficient, folate replete pregnant women, between 17 and 34 weeks gestation, a total dose of vitamin B_{12} of more than 1000 µg (approximately 8 µg/d) was associated with a reduction of plasma tHcy concentrations measured in late pregnancy. This evidence supports, for populations with a high prevalence of vitamin B_{12} deficiency, a public health message that vitamin B_{12} supplementation in addition to folic acid supplementation will improve vitamin status and lower homocysteine levels before and during pregnancy. The potential beneficial effects on foetal growth, neonatal development and future risk for type 2 diabetes and cardiovascular disease need to be investigated in a properly designed study.

ACKNOWLEDGEMENTS

The International Atomic Energy Agency, Vienna, Austria, provided financial support. We are grateful to the pregnant women for taking part in this study. We thank Dr KJ Coyaji, Director of the KEM Hospital, Pune, for providing research facilities. We thank Dr SS Naik, Mrs PC Yajnik, Ms LV Ramdas, Mr TM Deokar, Mr SD Chougule, Mr AB Gaikwad, and drivers for their invaluable contribution to the study.

AUTHOR DISCLOSURES

None of the authors had any financial or personal conflicts of interest associated with this manuscript.

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Original Article

Vitamin B_{12} and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B_{12} and high folate status

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低維生素 B_{12} 及高葉酸狀況的印度懷孕婦女，補充維生素 B_{12} 及葉酸與其血漿中同半胱胺酸濃度

母親若有維生素 B_{12} 缺乏及高同半胱胺血症，被預測會有較差的懷孕結果、胎兒可能肥胖及有胰島素抗性。在印度，極少數的開業醫生及決策者，領悟維生素 B_{12} 缺乏的廣泛存在。本研究調查在鄉村健康中心的產前診所及印度浦那市的轉診醫院中看診的 163 位懷孕婦女(86 位鄉村，77 位都市)，分別於孕期的 17、28 及 34 週時給予維生素補充劑，並測量血液中的維生素 B_{12}、葉酸及同半胱胺酸濃度。在納入研究時，有 80%的鄉村婦女及 65%的都市婦女有低維生素 B_{12} 濃度；但只有 2 位鄉村婦女有低葉酸濃度。在懷孕期間，有 85%的鄉村婦女及 95%的都市婦女接受葉酸補充；12%鄉村婦女及 84%的都市婦女接受維生素 B_{12} 補充。沒有接受補充劑的 17 位婦女中，在懷孕 17 週至 34 週的血漿維生素 B_{12} 及葉酸並沒有變化，但血中同半胱胺酸濃度則顯著增加(p<0.05)。只接受葉酸補充的 71 位婦女，在懷孕第 34 週時的平均同半胱胺酸濃度(8.4 μmol/L)，與沒有接受補充劑的懷孕婦女(9.7 μmol/L)相近；但是接受總劑量大於 1000 μg 維生素 B_{12} 的婦女(42 位，皆有接受葉酸補充)，至孕期第 34 週時，則有較低的同半胱胺酸濃度(6.7 μmol/L)。維生素 B_{12} 劑量增加，與降低血中同半胱胺酸濃度有相關；但葉酸劑量增加則與血中同半胱胺酸濃度無相關。在維生素 B_{12} 缺乏，但葉酸足夠的婦女，於懷孕後期給予維生素 B_{12} 補充劑與降低其血漿同半胱胺酸濃度有關連。

關鍵字：維生素 B_{12}、葉酸、同半胱胺酸、懷孕、印度人