Maternal and Child Health Journal (2019) 23:155–163
https://doi.org/10.1007/s10995-018-2605-z

Effect of Maternal Vitamin B12 Supplementation on Cognitive Outcomes in South Indian Children: A Randomized Controlled Clinical Trial

Susan Thomas[1] · Tinku Thomas[3] · Ronald J. Bosch[4] · Asha Ramthal[1] · David C. Bellinger[5] · Anura V. Kurpad[6,7] · Christopher P. Duggan[6,8] · Krishnamachari Srinivasan[1,2]

Published online: 12 July 2018

Abstract

Objectives To examine the effects of oral maternal vitamin B12 supplementation during pregnancy and early lactation on cognitive development in children. *Method* We studied 218 children born to mothers enrolled in a placebo-controlled, randomized trial of vitamin B12 supplementation during pregnancy through 6 weeks post-partum. Cognitive functions were assessed at 30 months using the Bayley Scales of Infant Development- 3rd edition (BSID III). The association of maternal sociodemographic characteristics, maternal biochemical status during pregnancy, birth weight and home environment with each sub-domain of BSID-III was examined using linear regression analysis. Separate multiple linear regression analyses for each of the BSID-III sub-domains with maternal trimester specific nutritional biomarker status was conducted. *Results* Children of mothers who received oral vitamin B12 supplementation had significantly higher scores on expressive language compared to children of mothers who received placebo ($\beta = 0.14$, $P = 0.03$). Children of mothers with elevated serum total homocysteine (tHcy) in the second and third trimesters of pregnancy had significantly lower scores on expressive language ($\beta = -0.18$, $P = 0.03$ and $\beta = -0.19$, $P = 0.02$, respectively) and gross motor domains ($\beta = -0.23$, $P = 0.008$ and $\beta = -0.30$, $P = 0.001$, respectively) of BSID-III adjusted for treatment arm and multiple confounders, compared with children whose mothers did not have elevated tHcy. *Conclusions for practice* Maternal B12 supplementation during pregnancy was associated with higher expressive language scores in children at 30 months. Elevated maternal tHcy levels during pregnancy had negative associations with expressive language and gross motor domains of BSID-III. Larger trials of maternal B12 supplementation are needed to confirm these findings.

Keywords Vitamin B12 supplementation · Elevated homocysteine levels · Cognition

✉ Krishnamachari Srinivasan
srinivas@sjri.res.in

1 Division of Mental Health & Neurosciences, St John's National Academy of Health Sciences, St John's Research Institute, Bengaluru, Karnataka 560034, India

2 Department of Psychiatry, St John's Medical College, Bengaluru, Karnataka, India

3 Division of Epidemiology, Biostatistics and Population Health, St John's Research Institute, Bengaluru, Karnataka, India

4 Department of Biostatistics, Harvard TH Chan School of Public Health, Boston, MA, USA

5 Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

6 Division of Nutrition, St John's Research Institute, Bengaluru, Karnataka, India

7 Department of Physiology, St Johns Medical College, Bengaluru, Karnataka, India

8 Division of Gastroenterology, Hepatology and Nutrition, Center for Nutrition, Boston Children's Hospital, Boston, MA, USA

Significance of the Study

Maternal nutrient status including vitamin B12 is important for the development of brain functions. Most of the studies that have examined the effect of maternal nutrient status on cognitive outcomes in children have either been observational in nature or conducted in developed countries. The present study using a randomized control design evaluated the effects of maternal B12 supplementation on cognitive outcomes in children from a resource poor setting and found an association between maternal B12 supplementation and some of the cognitive domains. The findings from the study need to be confirmed using larger trials of maternal B12 supplementation.

Introduction

Maternal nutrient status during pregnancy can have an influence on DNA methylation and is in turn likely to influence offspring neurodevelopment and cognitive performance (Dominguez-Salas et al. 2012; Georgieff 2007). Vitamin B12 is an important micronutrient involved in the development of brain during early life (Black 2008). Studies have shown that deficient Vitamin B12 status is linked to poor cognitive development in children (Kvestad et al. 2017; Venkatramanan et al. 2016; Strand et al. 2013). However, findings from studies that examined the association between maternal B12 status and childhood cognitive functions have been inconsistent. While two studies from developing countries reported association (Bhate et al. 2008; Rio Garcia et al. 2009), others did not find any associations between maternal vitamin B12 status or B12 intakes and childhood cognitive performance (Veena et al. 2010; Wu et al. 2012; Bonilla et al. 2012). Some of these studies were located in industrialized countries with largely well -nourished population (Bonilla et al. 2012), while others had small size and insufficient variation in vitamin B12 status (Wu et al. 2012) and used self-reported dietary B12 intake (Bonilla et al. 2012). In addition, the two studies that reported reduced cognitive functions in children of women with B12deficiency and low intakes were observational studies (Bhate et al. 2008; Rio Garcia et al. 2009), with residual confounding being a concern. Two recent systematic reviews on maternal micronutrient status and offspring cognitive functions suggested a need for randomized controlled trials especially in populations with high rates of micronutrient deficiencies (Leung et al. 2011; Veena et al. 2016).

We previously performed a randomized controlled trial of vitamin B12 supplementation from early in gestation through 6 weeks post-partum in South Indian pregnant women (Duggan et al. 2014). In a follow-up study of this cohort, we assessed cognitive outcomes in 178 infants using the Bayley Scales of Infant Development III at 9 months of age. While no significant effects of maternal B12 supplementation was observed on cognitive measures at 9 months, higher maternal plasma concentrations of total homocysteine (tHcy) (> 15.0 µmol/L) during the second trimester of pregnancy was significantly negatively associated with expressive language, and in the third trimester of pregnancy with expressive language and fine motor domains of BSID-III respectively (Srinivasan et al. 2017). The present study is a follow-up of the children from the same cohort examining the effects of maternal B12 supplementation on neurocognitive outcomes at 30 months, and whether the effects of higher maternal plasma concentrations of tHcy during pregnancy on cognitive outcomes in infants persists in early childhood.

Method

The parent randomized controlled trial was registered at clinicaltrials.gov as NCT00641862. This study was approved by the Institutional Ethics Committee, St. John's National Institute of Health Sciences, Bangalore and The Institutional Review Board of the Harvard T. H. Chan School of Public Health and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The study was conducted in urban Bangalore, India. The recruitment site was Hosahalli Hospital, a busy government hospital serving a poor community from urban Bangalore. Women and children who enrolled in the parent placebo-controlled, randomized trial of vitamin B12 supplementation during pregnancy and 6 weeks postpartum (Duggan et al. 2014) were eligible for inclusion in this follow-up study. Eligibility for the parent trial included women aged 18 years and older who had registered for prenatal care at or before 14 weeks gestational age (as judged by the date of the last menstrual period). Excluded were women with multiple gestations, chronic medical conditions (diabetes mellitus, hypertension, heart disease or thyroid disease) who anticipated moving out of the area before study completion, who tested positive for hepatitis B (HepBsAg), HIV or syphilis (VDRL) infections, and who were already taking daily vitamin supplements in addition to folate and iron. After obtaining informed consent, the women were randomly assigned to receive a daily oral dose of vitamin B-12 (50 µg) or a placebo identical in appearance (Cadila Pharmaceuticals) using a randomization list from 1 to 370 as prepared by the study biostatistician using permuted blocks of variable size in the parent trial. Compliance with the daily regimen was measured by research nurses counting unused supplements. Among the women who were administered vitamin B-12, the mean ± SD compliance rate was 69 ± 17%, and among those who were administered placebo, it was 70 ± 13% (P = 0.74) (Duggan et al. 2014).

All participants provided informed consent in their native language for the follow-up study. A study research assistant reviewed the baseline socioeconomic and demographic data that was collected upon initial entry into the trial. Trained research assistants measured maternal weight, height and infant weight, length and head circumference. The weights of the mothers were recorded using a digital balance (Salter's 9016; Tonbridge) to the nearest 100 g, and the heights were measured using a stadiometer to the nearest 0.1 cm.

Biochemical Assessments

Details of biochemical assays have been previously described (Duggan et al. 2014). Briefly, 10 mL of blood was obtained from the women by venipuncture at 12 weeks (baseline) and at 24 weeks and 33 weeks of pregnancy. The plasma and RBCs were separated and stored at − 80 °C until analysis. The plasma vitamin B12 was measured by the electrochemiluminescence method (Roche Diagnostics Mannheim, USA). The intraday and interday assay CVs for vitamin B12 were 0.54 and 2.44% respectively. Plasma tHcy and methyl malonic acid (MMA) were estimated by GC-MS (model 3800; Varian, Palo Alto, CA, USA). The interday assay CVs for tHcy and MMA were 5.04 and 5.57% respectively and the intraday assay CVs were 5.60 and 6.92%, respectively. Erythrocyte folate was measured by a competitive immunoassay with direct chemiluminescence detection on an automated immunonanalyzer (ADVIA Centaurs, Tarrytown, New York, USA). The intra-assay and inter-assay variabilities were 1.9 and 5.2%, respectively. The following cut-off values were used: low vitamin B12 as < 150 (pmol/L), elevated MMA as > 0.26 (μmol/L) and elevated tHcy as > 15.0 (μmol/L) as in the previous paper(Srinivasan et al. 2017).

The Bayley Scales of Infant Development, 3rd edition, (BSID-III) was used to assess infant neurodevelopment status at 30 months of age ± 2 weeks. The BSID-III assesses the developmental status of infants up to 42 months of age. The scales assess five domains: cognitive, language (receptive and expressive), motor (fine and gross), social-emotional, and adaptive (conceptual, social, practical). The latter two domains were not assessed as the BSID-III scale that assess them were deemed not culturally appropriate. Details concerning administration of BSID-III have been published previously (Srinivasan et al. 2017). Briefly, the test instructions were translated into local language and back translated and a standard script of the translated version was used during the administration of BSID-III. Testing was performed by a trained master's level psychologist in Hosahalli hospital and was carried out in a quiet room in a central location, with a parent or guardian present. As in our earlier assessment of neurocognitive outcomes at 9 months (Srinivasan et al. 2017), we used the raw scores from BDSI-III since age-specific norms are not available for Indian children. The scores were adjusted for the children's gestational age at birth.

The aspects of home environment were assessed using Bradley's home inventory for infants/toddlers (Bradley et al. 1989) at 1 year post- partum. This scale assesses parenting behaviour under six domains (responsivity, acceptance, organization, learning materials, parental involvement and variety of stimulation at home) and has been used in different cultures to study the impact of home environment on childhood cognitive abilities (Bradley et al. 1996).

Statistical Analysis

Continuous data were described using mean ± SD and categorical data using number (%). The demographic and baseline biochemical characteristics were compared between the treatment and the placebo groups using t tests and χ^2 tests. The association of maternal sociodemographic characteristics such as age, income, parity and maternal tHcy status during pregnancy, birth weight, home environment and treatment with each sub-domain of BSID-III was examined using linear regression analysis. A multiple variable regression was done for B12 treatment group (Vitamin B_{12} intervention vs placebo group) after adjusting for baseline B12 deficiency status which alone was found to be different between the groups. Since an association between elevated maternal tHcy levels and some of the cognitive outcomes was found in these infants at 9 months of age (Srinivasan et al. 2017), we re-examined the associations between each of the BSID-III sub-domains at 30 months of age and maternal trimester specific tHcy using multiple linear regression analysis. Similar to the approach used in our earlier study on cognitive outcomes in infant at 9 months, the intervention and placebo group were combined for this analysis. The analysis was adjusted for treatment group, income, parity, home environment, birth weight, sex, and maternal age. These variables were included as they showed a significant association with scores on BSID III on bivariate analysis. Regression coefficients (β) and corresponding 95% confidence interval (95% CI) are reported. The level of significance used for interpreting the data was $P < 0.05$. All data were analysed using SPSS for Windows, Version 22.0.

Results

Of the 366 women who were recruited in the parent trial, birth details were available for 256 newborns. 218 of these children were available for BSID-III assessment at 30

Fig. 1 : Flow chart of subject recruitment and randomization

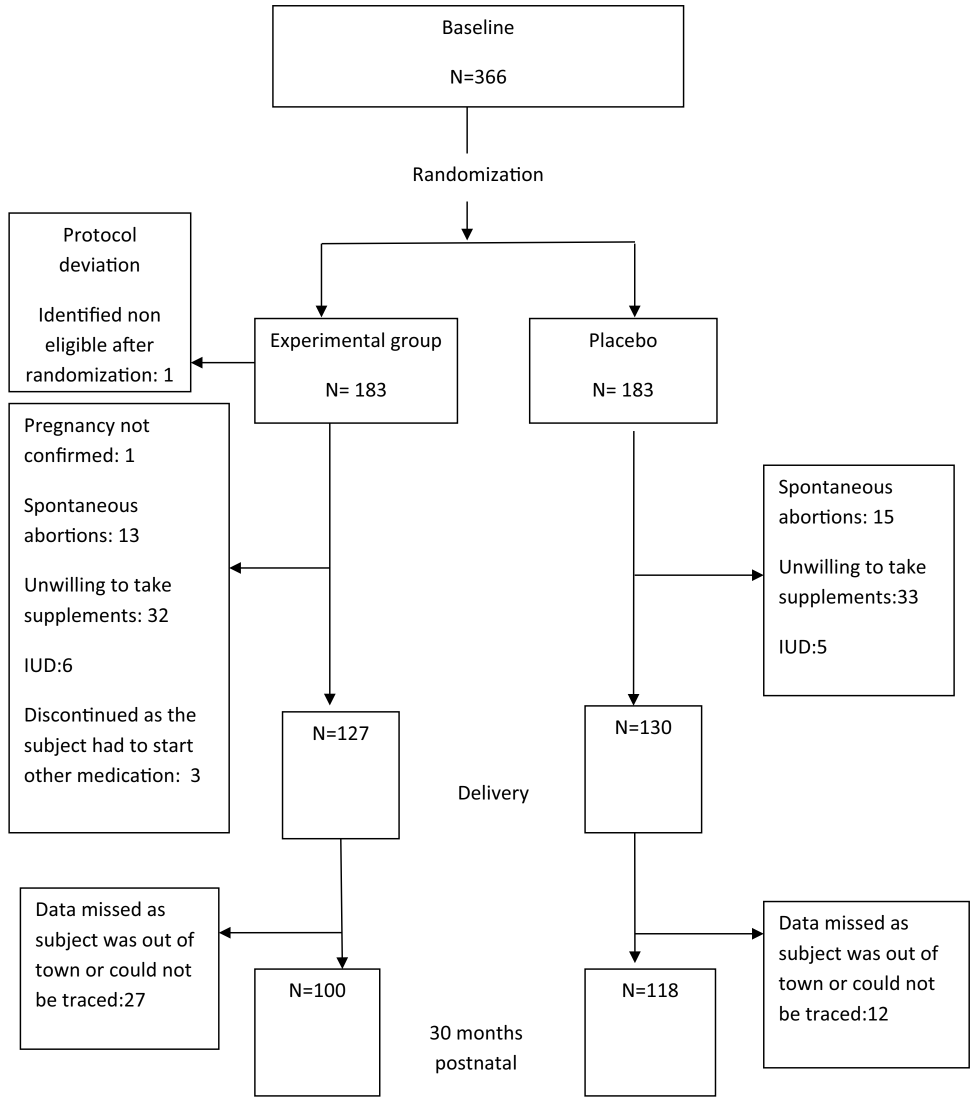

Table 1 Demographic characteristics of pregnant women whose infants underwent BSID III assessment at 30 months of age by study group

Parameters (N = 218)	Intervention group (N = 114) Mean ± SD or N (%)	Placebo group (N = 104)
Age, years	27.18 ± 3.93	26.80 ± 3.22
Level of education		
Up to middle school	26 (22.8)	19 (18.26)
High school	51 (44.7)	39 (37.5)
Post high school	23 (20.17)	18 (17.30)
Monthly household income, INR[a]	8313.13 ± 5480.85	7836.17 ± 4916.49
Mother employed	16 (14.03)	15 (14.42)
BMI, kg/m^2	20.90 ± 2.99	20.43 ± 3.72
Only child	58 (50.87)	74 (71.15)
Infant male sex	57 (50)	50 (48.07)
Infant birthweight	2.85 ± 0.46	2.84 ± 0.45
Scores on Bradly’s Home Inventory	37.07 ± 2.40	36.82 ± 2.24

[a]1 INR = 0.017 US dollars at time of study

Table 2 Baseline biochemical characteristics of 218 pregnant women whose infants underwent BSID testing at 30 months of age

Variables	Intervention group (N = 114)	Placebo group (N = 104)
Vitamin B12, < 150 pmol/L	44 (38.6)[b]	61 (58.6)[b]
MMA, > 0.26 µmol/L	83 (72.8)	81 (77.9)
tHcy, > 15.0 µmol/L	27 (23.68)	26 (25.0)
Erythrocyte folic acid, nmol/L[a]	424.64 ± 216.91	412.40 ± 185.50
Anemia, Hb < 11.0 g/dL	45 (39.5)	30 (28.8)

All data are N (%)

[a]Mean ± SD

[b]Significantly different at $p < 0.05$

months (Fig. 1). No differences were found in the socio demographic characteristics (Table 1) or baseline biochemical characteristics of the women whose children were available for neurocognitive evaluation at 30 months on BSID-III compared to children who were unavailable. The demographic and biochemical characteristics of the intervention and placebo groups are presented in Tables 1 and 2. The groups were comparable on demographic and biochemical characteristics with the exception of B12 status at baseline, with a significantly higher number of women with B12 deficiency in the placebo group (N = 61, 58.6%) when compared to the intervention group (N = 44, 38.6%) (Table 3).

The expressive language sub-domain scores on BSID, were significantly higher in children born to women in the vitamin B12 supplementation group compared to children of women who received placebo (36.01 (3.85) vs. 34.78 (4.49), P = 0.03) at 30 months (Fig. 2). This effect was also observed in the multiple linear regression analysis (Table 4), after adjusting for baseline B12 status. The scores on the other four BSID-III sub-domains at 30 months of age were comparable between the children born to women who received vitamin B12 supplementation during pregnancy and women who received placebo (Fig. 2).

The association of BSID-III scores with biochemical, sociodemographic and psychological characteristics, and birth weight of the child were further examined with linear regression (Table 3). All variables except parity were significantly positively associated with at least one of the BSID-III domain scores in the unadjusted analyses. Higher concentrations of plasma maternal tHcy levels in the second trimester was significantly negatively associated with expressive language (β = − 0.24, P = 0.001) and gross motor domain scores (β = − 0.25, P = 0.001). Elevated maternal tHcy levels in the third trimester was also significantly negatively associated with expressive language (β = − 0.22, P = 0.006) and gross motor domain scores (β = − 0.29, P < 0.000) .

Table 3 Bivariate regression of Bayley Scales of Infant Development in children at 30 months of age

Bayley subscale	Cognitive		Receptive language		Expressive language		Fine motor		Gross motor	
Bivariate analyses	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Treatment	0.04	(− 0.72, 1.35)	0.06	(− 0.80, 2.21)	**0.14**	(0.10, 2.36)	0.23	(− 0.34, 1.42)	0.01	(− 1.09, 1.31)
Income (Thousands of Indian Rupees)	0.02	(− 0.09, 0.12)	0.03	(− 0.19, 0.19)	**0.18**	(0.03, 0.25)	0.05	(− 0.06, 0.12)	0.11	(− 0.02, 0.21)
Parity	0.11	(− 0.20, 2.04)	0.03	(− 1.26, 1.98)	0.06	(− 0.67, 1.73)	0.00	(− 0.93, 0.98)	− 0.04	(− 1.60, 0.91)
Home Environment (total score)	0.12	(− 0.05, 0.46)	**0.15**	(0.01, 0.68)	**0.18**	(0.06, 0.61)	**0.22**	(0.11, 0.53)	0.05	(− 0.18, 0.40)
Birth weight	**0.22**	(0.69, 2.96)	**0.18**	(0.50, 3.77)	0.10	(− 0.33, 2.13)	0.08	(− 0.43, 1.52)	− 0.07	(− 1.97, 0.67)
Sex (female)	0.12	(− 0.20, 2.09)	**0.16**	(0.24, 3.53)	**0.17**	(0.26, 2.68)	**0.17**	(0.18, 2.09)	− 0.01	(− 1.34, 1.24)
Maternal age	**0.20**	(0.07, 0.36)	**0.15**	(0.02, 0.45)	0.07	(− 0.08, 0.24)	0.07	(− 0.06, 0.19)	− 0.07	(− 0.25, 0.08)
Elevated tHcy (> 15.0 µ mol/L)										
Trimester 1	− 0.09	(− 2.01, 0.39)	− 0.00	(− 1.79, 1.71)	− 0.06	(− 1.90, 0.74)	− 0.02	(− 1.163, 0.90	− 0.08	(− 2.24, 0.55)
Trimester 2	0.00	(− 1.79, 1.82)	− 0.00	(− 2.72, 2.62)	**− 0.24**	(− 5.06, − 1.26)	− 0.07	(− 2.28, 0.86	**− 0.25**	(− 5.40, − 1.35)
Trimester 3	− 0.02	(− 1.86, 1.48)	0.00	(− 2.55, 2.67)	**− 0.22**	(− 4.40, − 0.75)	− 0.07	(− 2.17, 0.80	**− 0.29**	(− 5.62, − 1.74)

Bold values indicate statistical significance at $p < 0.05$

Higher plasma concentrations of maternal tHcy levels in the second (β −0.18, P=0.03) and third (β −0.19, P=0.03) trimesters remained negatively associated with expressive language domain of BSID-III at 30 months in the adjusted analyses. Similarly, higher plasma concentrations of maternal tHcy in the second (β −0.23, P=0.008) and third trimesters (β −0.30, P=0.001) were negatively associated with gross motor skills domain of BSID-III at 30 months in the adjusted analyses.

Discussion

The major findings in this randomized controlled trial of maternal B12 supplementation and cognitive outcomes in children at 30 months of age were that children born to women who received oral B12 supplementation during pregnancy performed better on expressive language domain compared to children born to women who received placebo. In addition, in the group taken together as a whole, higher concentration of plasma maternal tHcy levels in the second and third trimester were negatively associated with expressive language and gross motor domain of BSID-III. This finding extends our earlier observation of an association between elevated maternal tHcy levels and expressive language and motor domains of the BSID-III when children were at 9 months of age (Srinivasan et al. 2017).

While some studies from low income countries have found an association with maternal B12 concentrations and B12 intakes and cognitive outcomes in children (Veena et al. 2010; Wu et al. 2012), to the best of our knowledge, ours is the first study using a randomized controlled design that examined the effects of oral maternal B12 supplementation during pregnancy on cognitive outcomes in children. In the present study, we found a modest effect of oral maternal B12 supplementation on expressive language domain of BSID-III at 30 months of age which is in contrast to the earlier report that did not find any effects of maternal B12 supplementation on any of the sub-domains of BSID-III when children were tested at 9 months of age (Srinivasan et al. 2017). There may be several reasons for this discrepant finding. First, our earlier study on cognitive outcomes at 9 months had a modest sample size where only 178 infants were available for cognitive assessments (Srinivasan et al. 2017). Secondly, the reliability and stability of scores of cognitive functions increase as the children get older (Shepard et al. 1998; Smith et al. 2001) making it difficult to reliably capture cognitive outcomes in early infancy (Marks et al. 2008).

We had earlier reported an association between elevated levels of maternal total homocysteine across all three trimesters and poorer cognitive functions on some of the sub-scales of BSID-III in infants at 9 months of age (Srinivasan et al. 2017). We now extend this finding with higher concentrations of plasma maternal tHcy levels in the second and third trimester of pregnancy continuing to be inversely associated with some of BSID-III scores in children at an older age (30 months). The effects of elevated maternal homocysteine levels on cognition of children are not well understood. A study of 320 Nepalese children, found that 5 year old children who had high levels of tHcy and MMA in infancy performed poorly on a neuropsychological test (the NEPSY), suggestive of a long term effect of hyperhomocystenemia on cognitive abilities (Kvestad et al. 2017). In addition, several studies especially in the elderly have reported an association between hyperhomocysteinemia and poorer cognitive abilities independent of serum B12 and folate levels

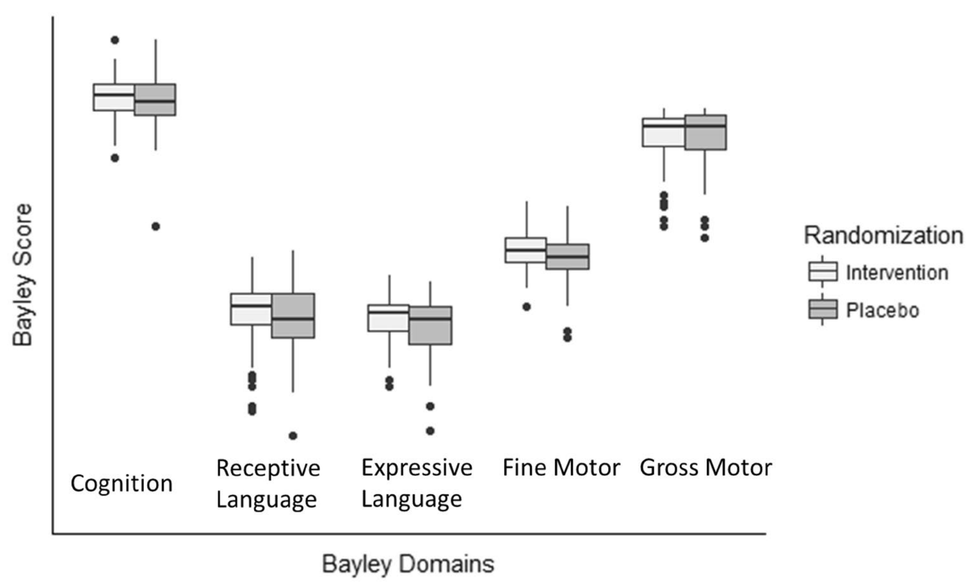

Fig. 2 BSID domain score at 30 months, by treatment group. Expressive language scores were higher in the children born to women in the vitamin B12 supplementation group (Mean (SD) for intervention group and placebo group were 36.01 (3.85) and 34.78 (4.49) respectively, P value=0.03

Table 4 Multivariate linear regression of Bayley Scales of Infant Development in children at 30 months of age

Bayley subscale	Cognitive		Receptive language		Expressive language		Fine motor		Gross motor	
Multiple variable analysis	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Treatment group[a]	0.05	−0.87, 1.69	0.13	−0.38, 3.15	0.18	0.17, 2.96	0.10	−0.41, 1.79	0.04	−1.15, 1.97
Elevated tHcy (> 15.0 μ mol/L)[b]										
Trimester 1	−0.08	(−2.14, 0.67)	0.03	(−147, 2.23)	−0.01	(−1.60, 1.36)	0.04	(−0.85, 1.50)	−0.06	(−2.27, 1.06)
Trimester 2	0.03	(−1.81, 2.67)	0.03	(−2.36, 3.44)	**−0.18**	(−4.70, −0.21)	0.02	(−1.67, 2.10)	**−0.23**	(−5.98, −0.93)
Trimester 3	0.01	(−1.90, 2.03)	0.04	(−2.12, 3.39)	**−0.19**	(−4.70, −0.35)	−0.07	(−2.56, 1.07)	**−0.30**	(−6.68, −1.85)

Bold values indicate statistical significance at $p < 0.05$

β regression coefficient, *95% CI* 95% confidence interval

[a]Adjusted for baseline B12 deficiency

[b]Adjusted for treatment arm, income, parity, home environment, birth weight, sex, and maternal age

(Miller et al. 2003; West et al. 2011). A systematic review of 111 studies (24 cohort studies, 18 randomized trials, 21 case-control studies, and 48 cross-sectional studies) on the effect of homocysteine on cognition in general population and in individuals with cognitive impairment found a positive association between cognitive decline and increased plasma tHcy levels (Setién-Suero et al. 2016). Lokk (2003) reported that the association between homocysteine levels and cognitive function was stronger than that of the levels of vitamin B12/folate in his review of studies on the association between vitamin B12, folate, homocysteine and cognition in the elderly.

The effects of higher plasma concentrations of maternal tHcy on expressive language and motor sub-domains of BSID-III were observed at both 9 months (Srinivasan et al. 2017) and in the present study at 30 months of age. Ars et al. (2016) examined the effect of prenatal maternal folate insufficiency, high concentrations of tHcy and low vitamin B12 levels on brain morphology (MRI), cognitive outcomes and psychological problems (Child Behavior Checklist (CBCL) in 256 6 to 8 year old Dutch children. They observed that children of women with high concentrations of prenatal homocysteine levels (> 9.1 µmol/L) had significantly lower IQ (7 points) and performed poorly on language and visuospatial domains compared to children of women with normal plasma concentrations of tHcy. However, they did not observe any associations between elevated tHcy levels and total brain volume on MRI. In a, double-blind randomized control trial of 79 infants (< 8 months) with biochemical signs of impaired vitamin B12 function, defined as a plasma tHcy cocnentration ≥ 6.5 µmol/L, vitamin B12 supplementation with 400 mg hydroxycobalamin intramuscularly improved motor functions and regurgitations along with Vitamin B12 status. (Torsvik et al. 2013). These studies demonstrate that maternal micronutrient status could have long term effects on specific cognitive domains. However, the underlying mechanism remains unclear.

The present study has several strengths. Few studies have examined the association between maternal B12 supplementation and cognitive outcomes in children in a resource poor setting where B12 deficiencies tend to be common. In the present study, nutritional biomarkers were estimated at each trimester of pregnancy. In addition, several possible confounders that included maternal and child characteristics and home environment were captured. The study also has several limitations. The sample size was modest and the attrition rate was relatively high. We did not measure maternal intelligence, which is known to impact child cognitive outcomes. In addition, we could not examine the association between current micronutrient status of the child and cognitive outcomes due to unavailability of blood samples in many of the participating children.

Our findings of associations between both maternal vitamin B12 supplementation and higher maternal plasma concentrations of tHcy and specific cognitive domains add to the growing literature on the effects of antenatal maternal nutrient status on neural development and cognitive performance in children and has important policy implications as longer term B12 supplementation of pregnant women and infants may result in more optimal cognitive performance. This approach may be cost effective as oral vitamin B12 supplements are relatively inexpensive. Further studies are needed to explore the underlying mechanisms to understand the associations between maternal B12 and tHcy status and cognitive outcomes in children.

Acknowledgements The present study was supported by Indian Council of Medical Research Grant 5/7/900/12-RCH Grant. The parent trial was supported by the Indian Council of Medical Research Grant 5/7/192/06-RHN and the US National Institutes of Health Grant R03 HD054123. CD was supported in part by NIH Grants K24DK104676 and 2P30 DK040561. The ongoing follow up study is being supported by the Norwegian Research Council (Grant No. 234495). There are no conflicts of interest. We thank Dr Nirmala for helping us obtain permission to conduct the study at Hosahalli Hospital. We thank Ms Vijaya P. and Ms Surekha S. for technical assistance. We are grateful to Ms Sarita Chowdhary for conducting the analyses of total homocysteine and methylmalonic acid and Ms Beena Bose for conducting the analyses of vitamin B12. Finally we thank all the participating women and their children.

References

Ars, C. L., Nijs, I. M., Marroun, H. E., Muetzel, R., Schmidt, M., Steenweg-de Graaf, J., & White, T. (2016). Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: The Generation R Study. *British Journal of Nutrition, 22*, 1–9.

Bhate, V., Deshpande, S., Bhat, D., Joshi, N., Ladkat, R., Watve, S., & Yajnik, C. (2008). Vitamin B12 status of pregnant women and cognitive function in their 9-yr-old children. *Food and Nutrition Bulletin, 29*, 249–254.

Black, M. M. (2008). Effects of vitamin B12 and folate deficiency on brain development in children. *Food and Nutrition Bulletin, 29*(2), S126–S131.

Bonilla, C., Lawlor, D. A., Taylor, A. E., Gunnell, D. J., Ben-Shlomo, Y., Ness, A. R., & Lewis, S. J. (2012). Vitamin B12 status during pregnancy and child's IQ at age 8: A Mendelian randomization study in the Avon longitudinal study of parents and children. *PLoS ONE, 7*, e51084.

Bradley, R. H., Caldwell, B. M., Rock, S. L., Ramey, C. T., Barnard, K. E., Gray, C., & Johnson, D. L. (1989). Home environment and cognitive development in the first 3 years of life: A collaborative study involving six sites and three ethnic groups in North America. *Developmental Psychology, 25*(2), 217–235.

Bradley, R. H., Corwyn, R. F., & Whiteside-Mansell, L. (1996). Life at home: Same time, different places—An examination of the HOME inventory in different cultures. *Early Development and Parenting, 5*(4), 251–269.

Del Rio Garcia, C., Torres-Sanchez, L., Chen, J., Schnaas, L., Hernandez, C., Osorio, E., & Lopez-Carillo, L. (2009). Maternal MTHFR 677C T genotype and dietary intake of folate and vitamin (B12): Their impact on child neurodevelopment. *Nutritional Neuroscience, 12*, 13–20.

Dominguez-Salas, P., Cox, S. E., Prentice, A. M., Hennig, B. J., & Moore, S. E. (2012). Maternal nutritional status, C (1) metabolism and offspring DNA methylation: A review of current evidence in human subjects. *Proceedings of Nutrition Society, 71*, (1), 154–165.

Duggan, C., Srinivasan, K., Thomas, T., Samuel, T., Rajendran, R., & Muthayya, S. (2014). Vitamin B12 supplementation during pregnancy and early lactation increases maternal, breast milk and infant measures of vitamin B12 status. *Journal of Nutrition, 144*(5), 758–764.

Georgieff, M. K. (2007). Nutrition and the developing brain: Nutrient priorities and measurements. *The American Journal of Clinical Nutrition, 85*, 614S–620S.

Kvestad, I., Hysing, M., Shrestha, M., Ulak, M., Thorne-Lyman, A. L., Henjum, S., & Strand, T. A. (2017). Vitamin B-12 status in infancy is positively associated with development and cognitive functioning 5 y later in Nepalese children. *The American Journal of Clinical Nutrition, 105*(5), 1122–1131.

Leung, B. M. Y., Wiens, K. P., & Kaplan, B. J. (2011). Does prenatal micronutrient supplementation improve children's mental development? A systematic review. *BMC Pregnancy and Childbirth, 11*(1), 12.

Lökk, J. (2003). Association of vitamin B12, folate, homocysteine and cognition in the elderly. *Scandinavian Journal of Nutrition, 47*(3), 132–138.

Marks, K., Glascoe, F. P., Aylward, G. P., Shevell, M. I., Lipkin, P. H., & Squires, J. K. (2008). The thorny nature of predictive validity studies on screening tests for developmental-behavioral problems. *Pediatrics, 122*, 866–868.

Miller, J. W., Green, R., Ramos, M. I., Allen, L. H., Mungas, D. M., Jagust, W. J., & Haan, M. (2003). Homocysteine and cognitive function in the Sacramento area Latino study on aging. *The American Journal of Clinical Nutrition, 78*, 441–447.

Setién-Suero, E., Suárez-Pinilla, M., Suárez-Pinilla, P., Crespo-Facorro, B., & Ayesa-Arriola, R. (2016). Homocysteine and cognition: A systematic review of 111 studies. *Neurosciences and Biobehavioral Reviews, 69*, 280–298.

Shepard, L., Kagan, S. L., & Wurtz, E. (1998). *Principles and recommendations for early childhood assessments*. Washington, D.C: The National Education Goals Panel.

Smith, T., Pretzel, R., & Landry, K. (2001). Infant assessment. In R. Simeonsson & S. Rosenthal (Eds.), *Psychological and developmental assessment of special children* (pp. 176–205). New York: Guilford press.

Srinivasan, K., Thomas, T., Kapanee, A. R., Ramthal, A., Bellinger, D. C., Bosch, R. J., & Duggan, C. (2017). Effects of maternal vitamin B12 supplementation on early infant neurocognitive outcomes: A randomized controlled clinical trial. *Maternal and Child Nutrition, 13*, e12325.

Strand, T. A., Taneja, S., Ueland, P. M., Refsum, H., Bahl, R., & Bhandari, N. (2013). Cobalamin and folate status predicts mental development scores in North Indian children 12–18 mo of age. *The American Journal of Clinical Nutrition, 97*, 310–317.

Torsvik, I., Ueland, P. M., Markestad, T., & Bjorke-Monsen, A. L. (2013). Cobalamin supplementation improves motor development and regurgitations in infants: Results from a randomized intervention study. *The American Journal of Clinical Nutrition, 98*, 1233–1240.

Veena, S. R., Gale, C. R., Krishnaveni, G. V., Kehoe, S. H., Srinivasan, K., & Fall, C. H. (2016). Association between maternal nutritional status in pregnancy and offspring cognitive function during childhood and adolescence; a systematic review. *BMC Pregnancy Childbirth, 16*, 220.

Veena, S. R., Krishnaveni, G. V., Srinivasan, K., Wills, A. K., Muthayya, S., Kurpad, A. V., & Fall, C. H. (2010). Higher maternal plasma folate but not vitamin B-12 concentrations during pregnancy are associated with better cognitive function scores in 9-to 10-year-old children in South India. *Journal of Nutrition, 140*(5), 1014–1022.

Venkatramanan, S., Armata, I. E., Strupp, B. J., & Finkelstein, J. L. (2016). Vitamin B-12 and cognition in children. *Advances in Nutrition, 7*(5), 879–888.

West, R. K., Beeri, M. S., Schmeidler, J., Mitchell, D. B., Carlisle, K. R., Angelo, G., & Silverman, J. M. (2011). Homocysteine and cognitive function in very elderly nondemented subjects. *The American Journal of Geriatric Psychiatry, 19*, 673–677.

Wu, B. T., Dyer, R. A., King, D. J., Richardson, K. J., & Innis, S. M. (2012). Early second trimester maternal plasma choline and betaine are related to measures of early cognitive development in term infants. *PLoS ONE, 7*, e43448.