Title: Effects of vitamin B complex supplementation during pregnancy on neonatal vitamin B_{12} status: evidence from a cluster randomized controlled trial

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Abstract

Purpose: Evidence about the effect of maternal vitamin B_{12} supplementation during pregnancy on offspring's vitamin B_{12} status is limited. The present study aimed to evaluate the impact of antenatal vitamin B complex supplementation on neonatal vitamin B_{12} status.

Methods: In an ongoing cluster randomized controlled trial conducted in three rural counties in northwest China, pregnant women <20 week of gestation were randomized to three treatment groups: blank control, iron supplements, or vitamin B complex supplements. All women were administered folic acid supplements during the periconceptional period. In a sub-study, we collected cord blood samples of 331 participants from the control or vitamin B complex groups in the Xunyi county from Jan.2017 to Dec.2017. Plasma concentrations of folate, vitamin B_{12}, and homocysteine were measured. Two-level linear mixed models were used to compare differences between groups.

Results: Compared with newborns whose mothers were in the control group, newborns of the vitamin B complex-supplemented women had significantly higher cord plasma vitamin B_{12} concentrations ($P = 0.001$) and lower homocysteine concentrations ($P = 0.033$). There was no significant difference in cord plasma folate concentrations between groups ($P > 0.05$).

Conclusions: Maternal vitamin B complex supplementation during pregnancy is effective in improving neonatal vitamin B_{12} status in rural northwest China.

Keywords: Antenatal vitamin B complex supplementation; Newborns; Vitamin B_{12} status; Cluster randomized controlled trial; Rural northwest China

Introduction

Vitamin B_{12}, also known as cobalamin, is a water-soluble vitamin that is mainly acquired from animal-source foods such as meat, poultry, eggs, seafood, and dairy products [1]. Together with folate, vitamin B_{12} is involved in the synthesis of purines and pyrimidines as well as the methylation of DNA, RNA and protein [2]. To prevent the occurrence of neural tube defects, folic acid (FA) supplements are widely recommended for women planning to become pregnant [3] and mandatory FA fortification programs have been implemented in numerous countries [4]. Vitamin B_{12} deficiency is common among women of childbearing age in parts of the world, however, was less studies than folate [5]. In a population-based cross-sectional study in Shaanxi province of northwest China, we reported that the prevalence of vitamin B_{12} deficiency among rural women aged 10-49 years was 52.0% [6], which is comparable to that of South Asia [7-10]. Moreover, another survey in this region indicated that only 16.8% of pregnant women took vitamin B_{12}-containing supplements before conception or during pregnancy [11].

Accumulating studies showed that suboptimal vitamin B_{12} concentrations during pregnancy is associated with neural tube defects [12], intrauterine growth retardation [13], as well as poor cognitive function [14] and high insulin resistance [15] in children. We hypothesized that antenatal vitamin B_{12} supplementation may be a cost-effective and feasible strategy to improve offspring's vitamin B_{12} status and short-term and long-term health outcomes. To our knowledge, few clinical trials have explored the effect of maternal vitamin B_{12} supplementation on offspring's biochemical indicators and most of them only focused on the South Asian populations [16-18].

A cluster randomized controlled trial in rural northwest China have been conducted to assess the protective effect of micronutrient supplementation during pregnancy on congenital heart disease and other adverse birth outcomes. Using data from the large trial, the present study aimed to examine whether vitamin B complex supplementation during pregnancy could be effective to affect neonatal folate, vitamin B_{12}, and homocysteine (Hcy) concentrations.

Methods

Study design and participants

This study was a biomarker sub-study of the ongoing cluster randomized controlled trial (registered at clinicaltrial.gov as NCT02537392) that aimed to investigate the effect of micronutrient supplementation during pregnancy on congenital heart disease and other adverse birth outcomes. This trial was conducted in three rural counties (Changwu, Bin and Xunyi) in northwest China since July 2015. Women who were aged 15-49 years and have already been pregnant for less than 20 weeks were invited to participate in this trial. Exclusion criteria at recruitment included 1) use of supplements containing vitamin B complex or iron for more than two weeks, 2) having given birth to children with congenital heart disease or other birth defects, and 3) having serious illnesses. As pregnant women were administrated by township maternal and child health workers in northwest China, a cluster randomized design, with a township as the unit of randomization, was used to minimize the risk of contamination of the intervention. The township clusters were randomized to the control, iron, or vitamin B complex groups with a 1:1:1 ratio before enrolment.

In accordance with the national policy for pregnant women in China, all women in this trial were provided daily 400 μg FA supplements during pre-pregnancy and early pregnancy by County Health Commission [19]. Women in the control group didn't receive any other micronutrient supplements. Women in the iron group received a tablet (Ferrous sulfate/tablet; Jinan Yongning Pharmaceutical Co., Ltd) containing 60 mg of iron, and those in the vitamin B complex group received supplements (Weikangfu/tablet; Sino-American Shanghai Squibb Pharmaceuticals Ltd) containing 2 mg of vitamin B_1, 2 mg of vitamin B_2, 2 mg of vitamin B_6, 2 μg of vitamin B_{12}, 5 mg of calcium pantothenate, 15 mg of nicotinamide (per tablet). Participants in both the iron and vitamin B complex groups were instructed to take one tablet daily from enrolment to delivery. All micronutrient supplements were commercially available during the period of this trial.

Pregnant women who were enrolled in the Xunyi county from Jan.2017 to Dec.2017 were further invited to participate in the biomarker sub-study. Women were excluded from the sub-study if they decided to deliver outside the Xunyi county or had twin births. Since this sub-study aimed to investigate the effect of vitamin B complex supplementation on cord plasma concentrations, women in the iron group were also excluded. Finally, cord blood samples were collected from 331 women.

The study was conducted according to the guidelines in the Declaration of Helsinki. Ethical approval was obtained from the ethics review committee of the Xi'an Jiaotong University Health Science Center (No.2012008). All participants provided written informed consent before their participation in the study.

Data collection

At enrollment, sociodemographic characteristics, including age, education, occupation, household wealth status and reproductive history, were collected by face-to-face interviews using a standardised and structured questionnaire. After removal of heavy clothing and shoes, height was measured to the nearest 0.1 cm with a stadiometer and weight was measured to the nearest 0.1 kg with an electronic scale. Accordingly, BMI was calculated as weight in kilograms divided by height in meters squared

The number of dispensed vitamin B complex supplements and those returned at each bimonthly antenatal check were record. Participants were also asked to report the use of FA supplements before conception and during pregnancy within one week after delivery. All the information was recorded by township maternal and child health workers through a web-based surveillance system.

Cord blood collection and laboratory analysis

Cord blood samples were collected from pregnant women at delivery using 5-mL EDTA-treated vacutainer tubes and kept on 4 °C. Plasma and buffy coat were separated within 24 h and stored at -80 °C until analysis. Plasma was analyzed within 12 months after collection by using the electro-chemiluminescence assay for folate and vitamin B_{12} and the enzymatic assay for Hcy. The intraassay CVs for folate, vitamin B_{12} and Hcy were 6.7%, 3.4% and 3.0%, and the interassay CVs were 8.1%, 7.0% and 7.3%, respectively.

Statistical analysis

The sample size in this sub-study was able to detect a 31.6% standard deviation unit difference between groups with a 2-sided type I error rate of 5% and 80% power. Household wealth index was constructed from an inventory of 15 household assets or facilities through principal component analysis, and this index was classified as tertiles: low (poorest), medium (middle-income) and high (richest) [20]. The compliance of vitamin B complex supplements was calculated by dividing the number of supplements consumed by the number of days from enrollment to delivery. Mean ± SDs or medians (25th, 75th percentiles) were used to describe normally or abnormally distributed continuous variables, respectively. Numbers (%) were used to describe categorical variables.

All analyses were conducted based on the intention to treat principle. All biochemical indicators were log-transformed before analyses. The cluster level baseline characteristics between groups were compared by using Wilcoxon two-sample tests. To adjust for clustering effect, two-level mixed models (township to level 2 and individual to level 1) were applied to assess the individual level differences between groups, including baseline characteristics, number of FA supplements consumed, and cord plasma concentrations of folate, vitamin B_{12}, and Hcy. Continuous variables and categorical variables were analyzed by using linear mixed models and generalized linear mixed models, respectively. Subgroup analyses according to baseline characteristics (gestational age at enrolment, household wealth index, parity and maternal age) were further conducted. Potential interactions between baseline characteristics and vitamin B complex supplementation were evaluated by including an interaction term in models.

All statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc.). Two sided $P < 0.05$ were considered statistically significant.

Results

Figure 1 shows the flow chart of this study. A total of 760 pregnant women in the Xunyi county were enrolled in the trial from January to December 2017. Of these participants, 392 were eligible for inclusion in the biomarker sub-study. Of these eligible participants, seven were lost to follow-up, 19 had a spontaneous or induced abortion, one ended her pregnancy with stillbirth, and 34 refused cord blood sampling. Ultimately, cord blood samples were available for 331 women, representing 84.4% of the 392 eligible participants.

Baseline characteristics were similar between the participants with or without cord blood. Among the participants with cord blood, the mean (SD) age was 26.4 (4.4) years, and 9.7% were educated beyond junior school, and 92.2% were farmers. Primiparous women made up 52.3% of the study sample. The mean gestational age at enrolment was 14.9 (4.6) and nearly one-third (29.6%) had a gestational age <12 wk. The mean (SD) height, weight and BMI at enrolment was 159.5 (4.9) cm, 55.5 (7.3) kg and 21.8 (2.8) kg/m^2, respectively (Supplemental Table 1). The individual level baseline characteristics were balanced between the participants in the control group and those in the vitamin B complex group (Table 1). There were four clusters in the control group and five clusters in the vitamin B complex group. The two groups were almost identical in terms of cluster level baseline characteristics, including number of villages, area, population, number of pregnancies, per capita gross domestic product and per capita net income (Supplemental Table 2).

The mean (SD) number of FA supplements consumed from three months before conception to delivery was 77 (52) in the control group and 85 (63) in the vitamin B complex group, which was similar between groups. The mean (SD) number of vitamin B complex supplements consumed from enrollment to delivery was 129 (76) in the vitamin B complex group (Table 2). The mean (SD) compliance with vitamin B complex supplementation was 69.5% (30.6%).

The intracluster correlation coefficients for cord plasma concentrations of folate, vitamin B_{12}, and Hcy were 0, 0.03, and 0.02, respectively. The effect of vitamin B complex supplementation on cord plasma concentrations is shown in Table 3. Newborns of mothers who received vitamin B complex supplements had higher cord plasma vitamin B_{12} concentrations (median: 218.1 pg/mL *v*. 183.7 pg/mL, $P = 0.001$) and lower Hcy concentrations (median: 15.7 μmol/L *v*. 18.1 μmol/L, $P = 0.033$) than did those in the control group. No significant difference was observed between groups in cord plasma folate concentrations ($P > 0.05$). There was no evidence of a differential effect of the intervention by baseline characteristics (gestational age at enrolment, household wealth index, parity and maternal age) on cord plasma concentrations of vitamin B_{12} and Hcy (Table 4).

Discussion

In this cluster randomized controlled trial of vitamin B complex supplementation during pregnancy, we found that newborns born to supplemented mothers had higher cord plasma vitamin B_{12}

concentrations and lower Hcy concentrations, indicative of improved vitamin B_{12} status.

Vitamin B_{12} acts as a cofactor in the remethylation of Hcy and in the formation of succinyl-CoA [21]. Therefore, vitamin B_{12} deficiency can lead to accumulation of Hcy and methylmalonic acid [21]. Poor vitamin B_{12} status, characterized as low vitamin B_{12} concentrations and elevated concentrations of Hcy and methylmalonic acid, has been thought to be highly prevalent among women of childbearing age, especially in South Asia where the population is predominantly vegetarian [7-10]. There are limited data on vitamin B_{12} status among Chinese women. A nutrition survey in Shaanxi province of northwest China found that the vitamin B_{12} concentrations of women aged 10-49 years was low and nearly half of rural women were vitamin B_{12} deficiency [6]. Given the high rate of vitamin B_{12} insufficiency and the pivotal role of vitamin B_{12} in fetal growth and development, several clinical trials of vitamin B_{12} supplementation during pregnancy and early lactation have been conducted in South Asia. The results from the Matlab Trial in Bangladesh showed that the prevalence of vitamin B_{12} deficiency among infants aged 6 months decreased by 11% after multivitamin supplementation (containing 2.6 μg/day of vitamin B_{12}) from 14 week of gestation to 3-month postpartum [16]. In the trial in India, when supplemented with 50 μg/day of vitamin B_{12} from <14 week of gestation to 6-week postpartum, infant at 6 weeks of age had higher vitamin B_{12} concentrations and lower Hcy and methylmalonic concentrations [17]. In another intervention study in Bangladesh, supplementation with 250 μg/day of vitamin B_{12} throughout pregnancy and 3-month postpartum increased vitamin B_{12} concentrations and lowered Hcy and methylmalonic acid concentrations in both newborns and infants at 3 months of age[18]. In our trial, pregnant women <20 week of gestation were allocated to receive or not to receive vitamin B complex supplements during pregnancy. The supplements used in this trial contained 2 μg of vitamin B_{12} per tablet. Even with a low dose of vitamin B_{12}, newborns responded to vitamin B complex supplementation with significantly higher cord plasma vitamin B_{12} concentrations and lower Hcy concentrations. The variation in the dose of vitamin B_{12}, the duration of supplementation and time point measured between studies made it difficult to compare with each other. However, all these findings suggested that taking vitamin B_{12}-containing supplements during pregnancy is an effective way to improve neonatal or infant vitamin B_{12} status in populations where vitamin B_{12} deficiency is common.

It is noteworthy that even with vitamin B complex supplementation, cord plasma vitamin B_{12} status in study population was poorer in comparison with those previously measured in other populations. The median cord plasma Hcy concentrations in the vitamin B complex group is much higher than the values of 6.2-11.0 μmol/L reported in Norway [22], the Netherlands [23] and Bangladesh [18]. A possible explanation for the poor vitamin B_{12} status among newborns was that pregnant women residing in this region are suffering from chronic malnutrition. Xunyi county is located in the southern Loess Plateau of Shaanxi Province and is one of state-level poverty-stricken counties [24]. The availability of animal products may be limited by poor natural environment and low economic level. In addition, genetic factors may also affect the vitamin B_{12} status of newborns. Methylene-tetrahydrofolate reductase (MTHFR) is responsible for the conversion of 5,10-methylene-tetrahydrofolate to 5-methyl- tetrahydrofolate, which is essential for the remethylation of Hcy [25]. The MTHFR 677C>T variant results in a thermolabile enzyme that has reduced activity in the synthesis of 5-tetrahydrofolate [25]. Accordingly, individuals with the MTHFR 677T allele have higher Hcy concentrations. The frequency of the MTHFR 677T allele in Shaanxi Province was reported to be 43.3% [26], which is at a high level globally [27]. Our findings suggested that the dose of 2 μg/day of vitamin B_{12} during pregnancy may be inadequate to ensure the acceptable vitamin B_{12} status of Chinese newborns. Additional studies are required to further explore the optimal dose of vitamin B_{12}.

Hyperhomocysteinemia, which refers to abnormally high homocysteine concentrations in the blood, has been considered as an independent risk factor for cardiovascular disease [28], cognitive impairment [29] and adverse pregnancy outcomes [30]. Our finding of maternal vitamin B complex supplementation during pregnancy lowering neonatal Hcy concentrations offered a potential strategy for primary prevention. To further confirm the effects of antenatal vitamin B complex supplementation on offspring's clinical outcomes, it would be necessary to conduct a long-term follow-up of our trial.

The main strength of the present study is that the design of randomized controlled trial has the ability to investigate causal association between maternal vitamin B complex supplementation and neonatal vitamin B_{12} status. However, our findings should be interpreted cautiously in light of some limitations. First, neither participants nor researchers were blinded. On the basis of the fact that the outcome indicators in this study were objective and the attrition rates between groups were similar (vitamin B complex group *v.* control group: 17.0% *v.* 14.2%, $P = 0.444$), we believed that the non-blinded design has a limited impact on estimated treatment effects [31]. Second, the unit of randomization was a township rather than an individual, which might have introduced a larger random error. To adjust for clustering effect, two-level mixed models were conducted in the present study. Fortunately, both the cluster and individual level baseline characteristics were shown to be similar. Third, except for vitamin B_{12} and Hcy, other functional biomarkers of vitamin B_{12} such as methylmalonic acid and holo-transcobalamin have not been measured. Fourth, blood samples beyond the newborn period have not been collected in this sub-study. Thus, the longer-term effect of vitamin B complex supplementation on offspring's vitamin B_{12} status remains unknown.

In conclusion, in the cluster randomized controlled trial in rural northwest China, we found that maternal vitamin B complex supplementation during pregnancy could be effective in improving neonatal vitamin B_{12} status. Our study provided valuable evidence for the development of public health policies on vitamin B_{12} supplementation during pregnancy.

Conflict of interest

The authors have no conflicts of interest related to this study to disclose.

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Table 1 Baseline characteristics of pregnant women in control and vitamin B complex groups.

	Vitamin B complex group (*n* =156)	Control group (*n* =175)	*P*[a]
Age (y)	26.8 ± 4.6	26.0 ± 4.1	0.140
<25 y	59 (37.8)	82 (46.9)	0.088
25-29 y	63 (40.4)	66 (37.7)	
≥30 y	34 (21.8)	27 (15.4)	
Education			0.975
Primary school or below	102 (65.4)	112 (64.0)	
Junior school	39 (25.0)	46 (26.3)	
Senior high school or above	15 (9.6)	17 (9.7)	
Occupation			0.930
Farmer	143 (91.7)	162 (92.6)	
Others	13 (8.3)	13 (7.4)	
Household wealth index	0.1 (1.4)	-0.1 (1.5)	0.477
Poor	46 (29.5)	60 (34.3)	0.585
Medium	50 (32.1)	64 (36.6)	
Rich	60 (38.5)	51 (29.1)	
Parity	0.5 ± 0.6	0.5 ± 0.5	0.691
Primiparous	82 (52.6)	91 (52.0)	0.979
Multiparous	74 (47.4)	84 (48.0)	

Gestational age at enrolment (wk)	14.7 ± 4.8	15.1 ± 4.4	0.777
<12 wk	46 (29.5)	52 (29.7)	0.554
≥12 wk	110 (70.5)	123 (70.3)	
Height (cm)	159.4 ± 5.0	159.7 ± 4.9	0.573
Weight at enrolment (kg)	55.1 ± 7.0	55.9 ± 7.7	0.352
BMI at enrolment (kg/m^2)	21.7 ± 2.5	21.9 ± 2.9	0.515

Values are means ± SDs or n (%).

[a]Differences between groups were assessed by using two-level linear mixed models for continuous variables or two-level generalized linear mixed models for categorical variables.

Table 2 Number of supplements consumed in control and vitamin B complex groups.

	Vitamin B complex group (n = 156)	Control group (n = 175)	P^a
Folic acid supplements[b]	85 ± 63	77 ± 52	0.461
<60	57 (36.5)	59 (33.7)	0.799
60-119	62 (39.7)	82 (46.9)	
120-179	15 (9.6)	20 (11.4)	
≥180	22 (14.1)	14 (8.0)	
Vitamin B complex supplements[c]	129 ± 76	--	--
<60	19 (12.2)	--	--
60-119	48 (30.8)	--	
120-179	42 (26.9)	--	
≥180	47 (30.1)	--	

Values are means ± SDs or n (%).

[a]Differences between groups were assessed by using two-level linear mixed model for continuous variable or two-level generalized linear mixed model for categorical variable.

[b]From three months before conception to delivery.

[c]From enrollment to delivery.

Table 3 Effect of vitamin B complex supplementation during pregnancy on cord plasma concentrations of folate, vitamin B_{12}, and Hcy.

	Vitamin B complex group (*n* = 156)	Control group (*n* = 175)	*P*[a]
Plasma folate (ng/mL)	8.2 (5.9, 12.7)	7.6 (6.2, 11.3)	0.455
Plasma vitamin B_{12} (pg/mL)	218.1 (159.7, 298.1)	183.7 (141.7, 229.9)	0.001
Plasma Hcy (µmol/L)	15.7 (11.5, 22.4)	18.1 (12.9, 25.4)	0.033

Hcy, homocysteine.

Values are medians (25th, 75th percentiles).

[a]Differences between groups were assessed by using two-level linear mixed models.

Table 4 Stratified analysis of the effect of vitamin B complex supplementation on cord plasma concentrations of vitamin B_{12} and Hcy.

	Vitamin B complex group (*n* = 156)	Control group (*n* = 175)	P^a	*P* interaction
Plasma vitamin B_{12} (pg/mL)				
Gestational age at enrolment				0.293
<12 wk	224.5 (165.8, 287.0)	175.8 (144.0, 235.7)	0.001	
≥12 wk	213.1 (159.4, 301.9)	184.6 (139.3, 227.2)	0.002	
Household wealth index				0.842
Poor	216.7 (160.2, 325.5)	174.9 (133.3, 219.0)	0.006	
Medium	212.3 (157.1, 301.9)	187.3 (142.9, 230.1)	0.011	
Rich	224.5 (167.2, 288.8)	187.3 (148.4, 233.8)	0.044	
Parity				0.347
Primiparous	232.7 (180.1, 336.4)	201.2 (149.3, 256.8)	0.012	
Multiparous	204.7 (147.4, 273.5)	159.7 (133.7, 216.3)	<0.001	
Maternal age				0.742
<25 y	209.4 (160.2, 301.9)	175.2 (136.7, 233.8)	0.020	
25-29 y	204.9 (152.5, 273.5)	188.7 (145.3, 221.7)	0.010	
≥30 y	250.3 (179.6, 349.1)	178.4 (143.3, 239.7)	0.014	
Plasma Hcy (μmol/L)				
Gestational age at enrolment				0.150
<12 wk	14.6 (11.2, 19.9)	18.3 (13.3, 29.1)	0.014	

≥12 wk	16.3 (11.8, 22.4)	18.1 (12.9, 24.8)	0.236	
Household wealth index				0.146
Poor	15.2 (12.2, 22.3)	21.2 (16.4, 27.1)	0.008	
Medium	15.3 (11.4, 23.3)	16.9 (13.2, 25.0)	0.211	
Rich	16.3 (11.0, 21.5)	13.8 (10.6, 22.6)	0.935	
Parity				0.696
Primiparous	15.1 (11.3, 21.5)	15.8 (11.8, 24.3)	0.147	
Multiparous	16.3 (12.2, 22.4)	20.8 (15.2, 27.0)	0.055	
Maternal age				0.863
<25 y	15.2 (12.1, 22.0)	18.5 (12.8, 27.6)	0.050	
25-29 y	15.5 (10.6, 22.4)	16.5 (13.3, 24.8)	0.216	
≥30 y	16.9 (11.5, 22.4)	20.8 (13.4, 24.1)	0.425	

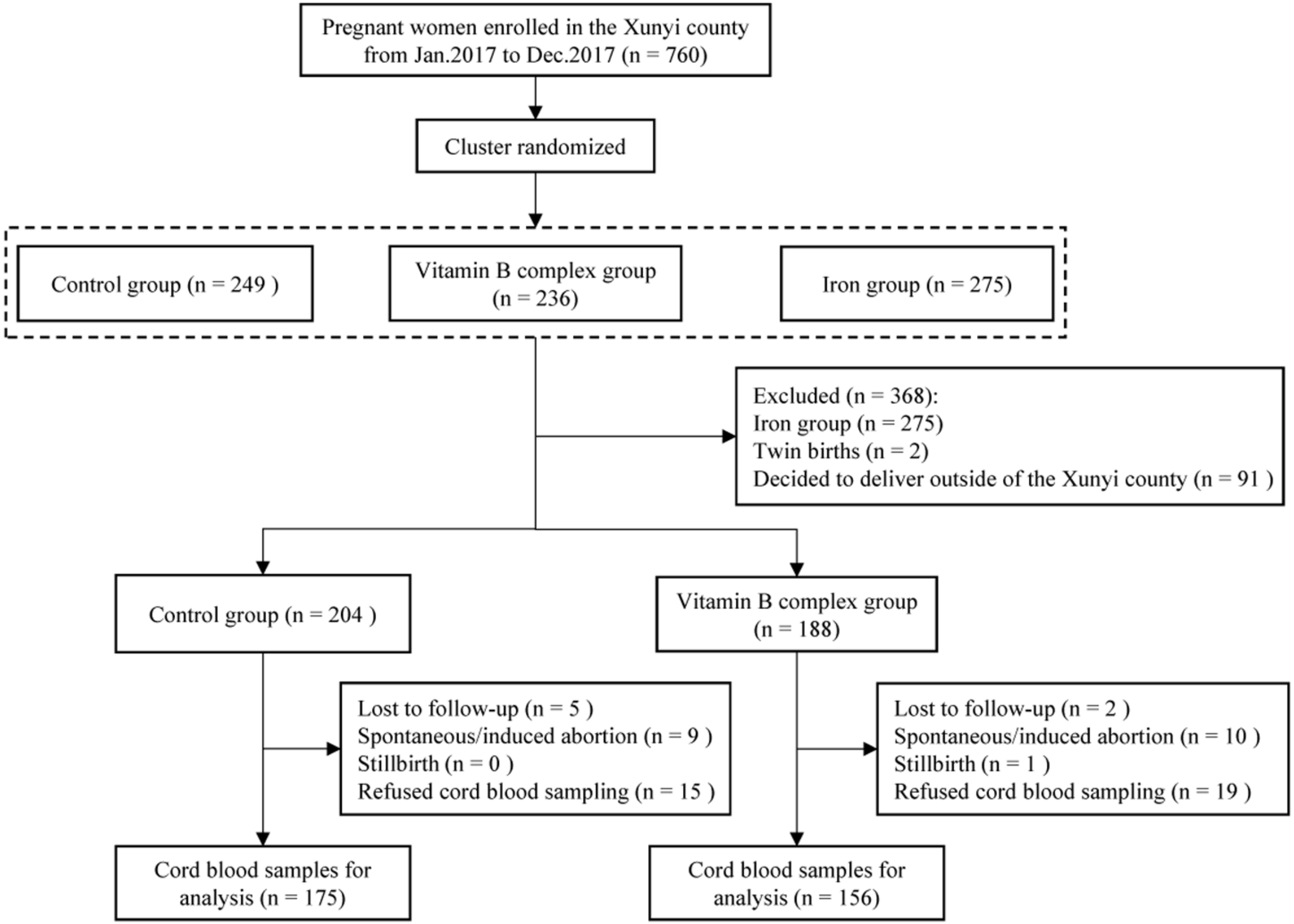
Hcy, homocysteine.

Values are medians (25th, 75th percentiles).

[a]Differences between groups were assessed by using two-level linear mixed models.

Figure legends

Fig.1 Study flow chart.


Pregnant women enrolled in the Xunyi county from Jan.2017 to Dec.2017 (n = 760)
Cluster randomized
Control group (n = 249)
Vitamin B complex group (n = 236)
Iron group (n = 275)
Excluded (n = 368):
Iron group (n = 275)
Twin births (n = 2)
Decided to deliver outside of the Xunyi county (n = 91)
Control group (n = 204)
Vitamin B complex group (n = 188)
Lost to follow-up (n = 5)
Spontaneous/induced abortion (n = 9)
Stillbirth (n = 0)
Refused cord blood sampling (n = 15)
Lost to follow-up (n = 2)
Spontaneous/induced abortion (n = 10)
Stillbirth (n = 1)
Refused cord blood sampling (n = 19)
Cord blood samples for analysis (n = 175)
Cord blood samples for analysis (n = 156)

Supplemental Table 1 Baseline characteristics of pregnant women with cord blood and those without cord blood.

	Cord blood collected (*n* = 331)	Cord blood not collected (*n* = 61)	P^a
Age (y)	26.4 ± 4.4	27.3 ± 4.3	0.169
<25 y	141 (42.6)	19 (31.2)	0.137
25-29 y	129 (39.0)	28 (45.9)	
≥30 y	61 (18.4)	14 (23.0)	
Education			0.053
Primary school or below	214 (64.7)	33 (54.1)	
Junior school	85 (25.7)	22 (36.1)	
Senior high school or above	32 (9.7)	6 (9.8)	
Occupation			0.270
Farmer	305 (92.2)	54 (88.5)	
Others	26 (7.9)	7 (11.5)	
Household wealth index	-0.001 (1.5)	0.004 (1.5)	0.562
Poor	113 (34.1)	18 (29.5)	0.722
Medium	107 (32.3)	24 (39.3)	
Rich	111 (33.5)	19 (31.2)	
Parity	0.5 ± 0.6	0.6 ± 0.6	0.506
Primiparous	173 (52.3)	30 (49.2)	0.516
Multiparous	158 (47.7)	31 (50.8)	

Gestational age at enrolment (wk)	14.9 ± 4.6	14.3 ± 5.5	0.355
<12 wk	98 (29.6)	25 (41.0)	0.070
≥12 wk	233 (70.4)	36 (59.0)	
Height (cm)	159.5 ± 4.9	158.5 ± 9.7	0.281
Weight at enrolment (kg)	55.5 ± 7.3	56.5 ± 7.8	0.367
BMI at enrolment (kg/m^2)	21.8 ± 2.8	22.9 ± 5.9	0.077

Values are means ± SDs or n (%).

[a]Differences between groups were assessed by using two-level linear mixed models for continuous variables or two-level generalized linear mixed models for categorical variables.

Supplementary Table 2 Baseline characteristics of clusters in control and vitamin B complex groups.

	Vitamin B complex group	Control group	*P*[a]
No. of clusters	5	4	--
No. of villages	12 (11, 12)	13 (9, 19)	1.000
Area (km^2)	42 (40, 74)	81 (55, 133)	0.623
Population	17125 (16858, 19321)	19180 (11072, 31405)	1.000
No. of pregnancies	156 (135, 187)	209 (134, 348)	0.806
Per capita gross domestic product (RMB yuan)	54400 (50180, 102750)	63076 (32444, 73371)	0.713
Per capita net income (RMB yuan)	10339 (9810, 11156)	9688 (9428, 11405)	0.540

Values are medians (25th, 75th percentiles).

[a]Differences between groups were assessed by Wilcoxon two-sample test.