Plan of Analysis

The effects of vitamin B12 supplementation in pregnancy and postpartum on growth and neurodevelopment in early childhood -a randomized double blind clinical trial

April 29, 2022
Version 1.0

Contents

1. Version history

Version number	Date	Comments
.5	November 2021	First draft of the plan of analysis. As described in the protocols to Nepal Health Research Council (NHRC) and The Norwegian Regional Research Ethics Board (REC). - Included the planned tables and figures for the main paper.
1.0	March 2022	Finalized draft of analysis: - Specified the main outcomes. - Included more detail on the statistical procedures. - Specified the statistical methods. - Included various changes to the wording and structure of the plan. - Included the term “key secondary outcomes”.

Nepal IRB: NHRC #: 253/2016
Norway IRB: REC #: 2016/1620
Acronym: VitaPreg
Other identifiers
Clinicaltrials.gov: *NCT03071666*
Universal Trial Number: *U1111-1183-4093*
Main funder: The Research Council of Norway, Centre of Excellence Scheme and the University of Bergen (UiB), Norway - Centre for Intervention Science in Maternal and Child Health (CISMAC; project number 223269).
Published protocol: Chandyo RK, Ulak M, Kvestad I, et al The effects of vitamin B12 supplementation in pregnancy and postpartum on growth and neurodevelopment in early childhood: Study Protocol for a Randomized Placebo Controlled Trial BMJ Open 2017;7:e016434. doi: 10.1136/bmjopen-2017-016434
Enrolment started: March 2017
Scheduled last day of follow up for the main outcomes: May 22, 2022
Planned date for breaking the randomization code: June 3, 2022
Submittable manuscript: June 25, 2022

2. Research question and project synopsis

Research question: Does daily supplementation with vitamin B12 during pregnancy and postpartum improve growth and development in infants.

Scientific basis: Globally, vitamin B12 deficiency is one of the most common micronutrient deficiencies. The primary, dietary sources of vitamin B12 are animal-derived foods. Vitamin B12 is crucial for normal cell division and differentiation, and necessary for the development and myelination of the central nervous system. Deficiency is associated with impaired fetal and infant growth as well as impaired neurodevelopment. In this study, we investigate the effect of maternal daily oral vitamin B12 supplementation from early pregnancy to 6 months postpartum on the neurodevelopment and growth of their children. We will also measure the effect of B12 supplementation on several other outcomes.

Study design: Individually randomized double-blind placebo controlled trial in pregnant Nepalese women at risk of vitamin B12 deficiency. The participants were randomized using a computer-generated sequence in a 1:1 ratio. Treatment allocation was concealed.

Study participants and site: 800 pregnant women from the Bhaktapur district in Nepal who were enrolled as early as possible, and no later than in week 15 of pregnancy.

Intervention: Daily administration of 50 µg of vitamin B12 (cyanocobalamin) from enrollment until 6 months after birth.

Comparator: Placebo, identical to the vitamin B12 supplement.

Primary outcomes: (i) neurodevelopment in children measured at 6 and 12 months of age (ii) attained, linear growth in children measured at 12 months of age.

Relevance for programs and public health: The results from this study can inform new dietary guidelines for fertile women in low- and middle-income countries.

3. Primary objectives

To measure the effect of 50 µg vitamin B12 supplementation daily in pregnancy and postpartum on:

- The Bayley Scales of Infant and Toddler Development, 3rd ed. (Bayley-III) measured at 6 and 12 months of age.
- Linear growth expressed as the length for age z-scores measured at 12 months of age.

3.1 Primary outcome neurodeveopment

- The Bayley-III cognitive composite score at 6 and 12 months.

3.2 Primary outcome growth

- Length for age z-scores at 12 months.

4. Secondary objectives

We will also report the effect of the intervention on several secondary outcomes related to growth, neurodevelopment, and pregnancy. These outcomes, categorized into *key* secondary outcomes and *other* secondary outcomes, are listed below:

4.1. Key secondary outcomes: neurodevelopment

- Bayley-III language and motor composite scores at 6 and 12 months, to include:
    - Language
        - Expressive
        - Receptive
    - Motor
        - Fine
        - Gross
- The Test of Infant Motor Performance Scale (TIMP) at 75 days, total score.

4.2. Key secondary outcomes: growth

- Infant's z-scores weight for age and weight for length at 6 and 12 months.
- Infant weight in grams at 6 and 12 months.
- Infant's z-scores length for age at 6 months.
- Infant length in centimeters at 6 and 12 months.

4.3. Key secondary outcomes: pregnancy

- Birth weight in grams.
- Proportion of infants born weighing less than 2,500 g and 2,000 g.
- Proportion of infants born weighing more than 4,000 g.
- Gestational length (according to the first estimated ultrasound gestational age) in cm/weeks.
- Proportion of infants born before 37 weeks of gestation.

4.4. Other outcomes

4.4.1. Pregnancy related

- Risk of pregnancy related complications, including:
    - Fetal loss
    - Hospitalization
    - Morbidities (Gestational diabetes, preeclampsia / eclampsia, and others)
    - Still birth
    - Medical termination
    - Neonatal death
    - Congenital anomality

4.4.3. Biomarkers

- Plasma concentrations of cobalamin, total homocysteine, and methylmalonic acid.
- The combined indicator of vitamin B12 (CB12) which is calculated from the concentrations of the biomarkers mentioned above. Briefly, CB12 is proportional to

the plasma cobalamin concentration divided by the product of total homocysteine and methylmalonic acid concentrations.
- End study hemoglobin concentration.

4.4.4. Adverse events

- Incidence of adverse events in supplemented pregnant women, including:
    - Vomiting within 30 minutes after supplement was ingested
    - Nausea within 30 minutes after supplement was ingested
    - Others

4.5. Assesment of compliance

- Assessment of number of tablets (placebo or intervention) taken during intervention period (early pregnancy – 6 months postpartum).

4.5. Predefined subgroup analyses

All outcomes listed above will be presented separately in those with "low" and "high" vitamin B12 status at baseline. We will use the CB12 to define vitamin status by dichotomizing the CB12 at the 33rd percentile.

We will also consider investigating the effect of vitamin B12 supplementation in other subgroups of the data (*post hoc* subgroup analyses). These results will not be presented in tables but within text (in the results section). These exploratory, hypothesis generating analyses will be undertaken to ensure that we do not overlook any potential, relevant effects of vitamin B12 supplementation on important outcomes in certain subgroups.

5. Planned tables and figures

5.1 Tables

Table 1: Baseline characteristics, stratified by placebo and vitamin B12 groups, will include variables collected before supplementation, including:

- Demographics.
- Health.
- Biochemical (vitamin B12 status at study enrolment).

Table 2: Infant outcomes (including the main outcomes):

- Neurodevelopment.
- Growth.
- Biochemical (vitamin B12 status at 6 and 12 months in a subgroup of the children).

Table 3: Pregnancy and safety outcomes, including:

- Birth outcomes.
- Maternal morbidity.
- Neonatal outcomes.
- Biochemical (vitamin B12 status at end of pregnancy and 6 months postpartum in a subgroup of the women).

Supplemental tables

Table S1: Baseline features by study group and by CB12 concentration dichotomized at the 33rd percentile).
Table S2: Compliance.
Table S3: Adverse events.

5.2. Figures

Figure 1: Trial flow-chart.
Figure 2: Primary outcomes and key secondary outcomes by CB12 concentration (dichotomized at the 33rd percentile) presented in two forest plots.

6. Statistical analyses

Our primary analysis will use an intention-to-treat approach where all pregnant women (or mother-infant pairs) who were randomized into the placebo or vitamin B12 arms of the trial and whose primary infant-outcome is known will be included.

Baseline maternal characteristics will be presented in a table stratified by intervention arm (Table 1). In addition, we will present these same maternal characteristics according to study group, stratified further into two predefined subgroups (CB12 dichotomized at the 33rd percentile) (supplementary Table S1).

We will use the same approach to analyze the effect on the primary outcomes and the key secondary outcomes.

All the listed outcomes (neurodevelopment and growth) are expected to be normally distributed. We will present differences in means for continuous outcomes and differences in proportions for the dichotomous outcomes. For these analyses, we will use Students t-tests, generalized linear, and mixed effect models with identity link function of the binominal family distribution (for differences in proportions) and of the Gaussian distribution family (for differences in means).

If the continuous variables are not normally distributed, we will transform these variables before analyses (most likely by log transformation).

We will present the main effect measure estimates as mean differences (or differences in proportions) with corresponding confidence intervals and p-values.

If relevant between-group baseline differences in any of the variables are observed (Table 1), we will consider adjusting the effect estimates for these potential confounding variables in the regression models and present the adjusted effect in the main text of the manuscript**.** For the subgroup analyses, we will adjust for variables expected to be associated with the outcomes and where there are baseline differences according to CB12 stratification (supplementary Table S1). In these sub-group analyses, we will include interaction terms to test the statistical significance of the effect modifier.

Handling of missing values: In the intention-to-treat analyses, observations with missing values will be excluded (listwise deletion). In other words, cases with missing exposure or outcome variables will not be used. If one of the two Bayley-III measurements (6 and 12

months) is missing we will not exclude the child from the analyses but will include only the valid measurement.

6.1. Other analyses that will be considered

We will consider exploring Instrumental Variable Analyses (IVA) in an attempt to shed light on what may be a better estimate of the intrinsic effect of vitamin B12 in pregnancy, i.e. the efficacy of vitamin B12 supplementation had it been given to all women (randomized to the vitamin B12 arm) in the scheduled doses and intervals. In our IVA, the random allocation will be the instrument and actual amount of vitamin B12 administered to the women during pregnancy and postpartum will be the exposure variable (supplementary Table S2: Compliance). We will also consider simple per protocol analyses in which patients who received less than for example 50% of the projected doses will be excluded from the analyses.