The effect of vitamin B12 supplementation during pregnancy on infant growth and development in Nepal: a community-based, double-blind, randomised, placebo-controlled trial

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Summary

Background Vitamin B12 is required for healthy infant growth and development, but low and marginal vitamin B12 status is endemic in low-income and middle-income countries. We aimed to measure the effect of vitamin B12 supplementation from early pregnancy until 6 months post partum on infant growth and neurodevelopment.

Methods In this community-based, double-blind, placebo-controlled trial, we randomly assigned (1:1) 800 pregnant women (aged 20–40 years) who were up to 15 weeks pregnant—recruited from home visits and outpatient departments at three hospitals in Nepal—to daily supplementation with 50 µg oral vitamin B12 or placebo until 6 months postpartum. Independent scientists generated the list that linked allocation to participants' study identification number. Participants were masked to group assignment and all investigators were masked until data cleaning was completed. The primary outcomes were length-for-age Z score (LAZ) at age 12 months and the cognitive composite score of the Bayley Scales of Infant and Toddler Development (3rd edition) at age 6 months and 12 months. The primary and secondary outcomes, including adverse events, were assessed in the intention-to-treat population, for all participants with available outcome data. This trial is registered with ClinicalTrials.gov, NCT03071666.

Findings 800 eligible pregnant women were enrolled in the trial between March 28, 2017, and Oct 15, 2020, with 400 women randomly assigned to each group. Follow-up was completed on May 18, 2022. At baseline, 569 (71%) of 800 women had plasma vitamin B12 indicating low or marginal status (<221 pmol/L). We found no effect of vitamin B12 on the primary outcomes. The mean LAZ at age 12 months were –0·57 (SD 1·03) in the B12 group and –0·55 (1.03) in the placebo group (366 infants in the vitamin B12 group *vs* 363 infants in the placebo group) with a mean difference of –0·02 (95% CI –0·16 to 0·13). The mean cognitive composite scores were 97·7 (SD 10·5) in the B12 group and 97·1 (10·2) in the placebo group, with a mean difference of 0·5 (95% CI –0·6 to 1·7) measured in 364 and 361 infants. Stillbirths or infant deaths occurred in three (1%) of 374 women in the vitamin B12 group and nine (2%) of 379 women in the placebo group.

Interpretation Although vitamin B12 deficiency was prevalent in our study population and vitamin B12 supplementation from early pregnancy substantially improved vitamin B12 status, supplementation did not improve infant growth or neurodevelopment. Our findings support the current WHO recommendations of no routine vitamin B12 supplementation during pregnancy.

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Introduction

Many children from low-income and middle-income countries (LMICs) do not reach their growth and developmental potential.[1] Vitamin B12 is required for typical growth and development, but low and marginal vitamin B12 status is endemic in many LMIC settings.[2,3] As an enzymatic cofactor for the transmethylation of homocysteine to methionine by methionine synthase and the rearrangement of methylmalonyl-coenzyme A (CoA) to succinyl-CoA in propionate metabolism by methylmalonyl-CoA mutase, vitamin B12 is essential for multiple physiological processes including: DNA methylation and histone modification; cell differentiation and growth; energy metabolism; and myelinisation of the CNS.[3,4] Although the importance of vitamin B12 throughout the lifecycle is recognised, it is especially important during pregnancy and early infancy. Failure to thrive, atypical neurological function, delayed development, and macrocytic anaemia are typical manifestations in infants with severe clinical vitamin B12 deficiency.[5,6] Results from several population-based studies show that even marginal vitamin B12 deficiency is associated with adverse pregnancy outcomes,[7] impaired infant growth, and poor neurodevelopment in early childhood.[8–10]

Research in context

Evidence before this study

We searched PubMed and Web of Knowledge on June 20, 2022 for studies published up to this date on vitamin B12, growth, and neurodevelopment using the search terms "Vitamin B_{12} "[MeSH], "B12"[Text Word], "cobalamin*" [Text Word] as well as "Clinical Trial"[Publication Type], "trial*"[Text Word], "supplement*"[Text Word], "Dietary Supplements"[MeSH], "Treatment Outcome"[MeSH], and "Vitamins/administration and dosage"[MeSH Terms]. We only included randomised controlled trials that reported the effects of supplementing vitamin B12 during pregnancy. Our searches identified seven publications from three studies on supplementation during pregnancy, five publications on supplementation during early childhood, and one meta-analysis. The trials during pregnancy were conducted in India (two studies) and Bangladesh (one study), with vitamin B12 doses of 50 µg, 2 µg, and 250 µg, respectively. The trial in rural Bangalore, India, found an effect of vitamin B12 supplementation on expressive language at age 30 months; however, no effect was observed on other domains or at other times during follow-up. The trial in Pune, India, found higher cognition and language scores at age 2 years in children of mothers who had taken vitamin B12 supplements before pregnancy than children of mothers who had not taken vitamin B12 supplements. Common to these studies were the relatively small sample sizes, ranging from 82 to 366 women and that growth and neurodevelopment were secondary outcomes. Meta-analysis results reported vitamin B12 deficiency was associated with low birthweight (adjusted risk ratio 1·15 [1·01–1·31]) and preterm birth (1·21 [0·99–1·49]). We also searched the registry platform ClinicalTrials.gov on June 20, 2022 and identified two relevant protocols on the effect of vitamin B12 supplementation during pregnancy, none of which has been published yet. Publications from 2021 and 2022, particularly targeting the Indian subcontinent, make a strong argument for public action to improve vitamin B12 status in women of childbearing age, particularly during pregnancy.

Added value of this study

To our knowledge, this is the first randomised clinical trial in pregnant women designed to measure the effect of vitamin B12 supplementation on infant growth and neurodevelopment. More than two-thirds of the study population had low or marginal vitamin B12 status at enrolment. Vitamin B12 supplementation during pregnancy and postpartum had a substantial effect on the direct and functional biomarkers of vitamin B12 status in the pregnant women and their infants. Supplementation did not, however, improve infant growth and neurodevelopment.

The prevalence of spontaneous abortion, low birthweight, preterm delivery, stillbirth, and neonatal deaths reported in our study is similar to the prevalence in the peri-urban population of Nepal. We found no effect of vitamin B12 supplementation on these outcomes, indicating the dose of 50 µg of vitamin B12 during pregnancy is generally safe in terms of pregnancy outcomes. A negative effect on early motor performance was observed, however, giving reasons for caution related to vitamin B12 supplementation during pregnancy.

Implications of all the available evidence

There was a high prevalence of low and marginal vitamin B12 status among pregnant women in our study population. Oral supplementation of vitamin B12 (50 µg per day) for 1 year starting in early pregnancy substantially improved vitamin B12 status in both mothers and infants but did not improve infant growth and neurodevelopment during the first year of life. The implications of improved vitamin B12 status for other outcomes merit further investigation.

Our findings, in conjunction with the scarcity of previous studies, support the current WHO recommendations of no routine vitamin B12 supplementation during pregnancy.

Findings from three randomised controlled trials (RCTs) indicate positive effects of vitamin B12 supplementation starting before or during early pregnancy on child health and neurodevelopment in the first 2–3 years of life.[11–14] These findings, however, do not suffice to change the current recommendations on routine vitamin B12 supplementation in pregnant women from LMICs, which is in line with the current WHO antenatal pregnancy guidelines.[15] Despite the scarcity of evidence and current public health recommendations, many argue for widespread and high-dose supplementation of vitamin B12 before and during pregnancy.[16,17]

We aimed to measure the effect of daily vitamin B12 supplementation from early pregnancy (<15 weeks' gestation) until 6 months post partum on infant growth and neurodevelopment. The primary outcomes were linear growth attained at age 12 months and neurodevelopment measured with the cognitive composite score of the Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III) at age 6 months and 12 months. We ensured that the baseline vitamin B12 status and the metabolic effect of supplementation were well characterised in both women and infants. Describing the metabolic response to supplementation provides an indication of intervention adherence and the intracellular demand for vitamin B12.[18] Moreover, we targeted a population in whom we have previously described poor vitamin B12 status[19] and high rates of stunting,[20] and for whom maternal and infant vitamin B12 status were positively associated with child growth and neurodevelopment.[8,21]

Methods

Study design and participants

This is a community-based, double-blind, randomised, placebo-controlled trial, conducted in Bhaktapur

municipality and surrounding areas in Nepal.[22] The ethical review boards in Nepal (NHRC 253/2016) and Norway (2016/1620/REK vest) approved the study. An independent data and safety monitoring board continuously oversaw severe adverse events and undertook an interim analysis when 100 infants had reached age 10 weeks.

Pregnant women aged between 20 and 40 years who were up to 15 weeks pregnant, as assessed by last menstruation period, were recruited from home visits and gynaecological outpatient departments at three hospitals in the study area and invited for participation. Pregnancy and gestational age were further confirmed by ultrasonography. Additional inclusion criteria were living in and planning to reside in the area for the next 2 years and consenting to participate. Pregnant women taking or planning to take dietary or multivitamin supplements containing vitamin B12 were excluded. Pregnant women with acute or chronic illness, severe anaemia, high-risk pregnancy, or a BMI lower than 18·5 kg/m² or higher than 30·0 kg/m² were also excluded. Each pregnant mother provided informed written consent, preferably in the presence of the other parent to reduce the risk of withdrawal of consent. The full protocol is in the appendix (pp 13–48).

See Online for appendix

Randomisation and masking

Eligible pregnant women were randomly assigned (1:1) to receive supplements with or without vitamin B12 in blocks of eight using a computer-generated randomisation list. The supplements, with or without vitamin B12 (50 µg), were identical in taste and appearance. The rationale for the chosen dose is given in the appendix (p 2). Allocation was concealed, and the

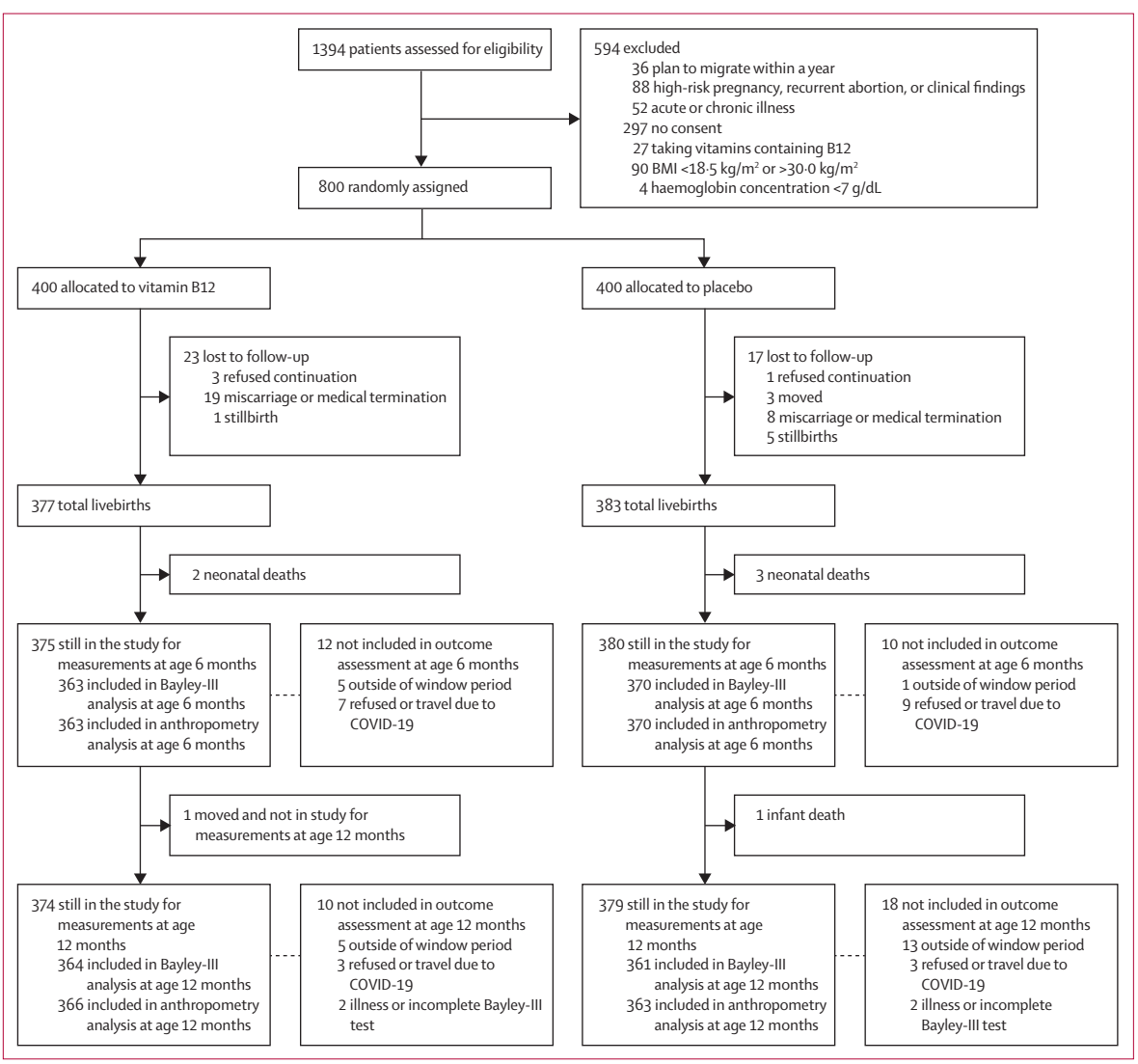

Figure 1: **Trial profile**
Bayley-III=Bayley Scales of Infant and Toddler Development, 3rd edition.

participants were linked to the study groups through an identification number printed on the supplement labels. The list that linked this identification number to the randomisation code was only available to the producers of the supplements and kept with two individuals who were otherwise not involved in the study. One of these individuals generated the randomisation list, and the other labelled the intervention packages according to the instructions given by the producers. These individuals also interacted with the data and safety monitoring board when the randomisation code was needed for the interim analysis. None of the study investigators had access to this list until data collection and data cleaning of the primary outcomes were completed.

	Vitamin B12 (n=400)	Placebo (n=400)
Age, years	27·7 (3·8)	27·5 (4·2)
Gestational week at enrolment*	11·0 (2·8)	11·1 (2·8)
History of previous abortion or miscarriage	73 (18%)	60 (15%)
Folic acid supplementation	287 (72%)	278 (70%)
Iron supplementation	76 (19%)	69 (17%)
Calcium supplementation	74 (19%)	68 (17%)
BMI, kg/m²	23·8 (3·0)	23·6 (3·0)
BMI >25 kg/m²	141 (35%)	128 (32%)
Vegetarian	4 (1%)	4 (1%)
Completed secondary school or above (≥11th grade at age 17–18 years)	229 (57%)	226 (57%)
Salaried job (private or government sector)†	117 (29%)	121 (30%)
Nuclear family (two generations or less)	145 (36%)	133 (33%)
Family own land	262 (66%)	277 (69%)
Family resides in rented house	102 (26%)	96 (24%)
Kitchen and bedroom in the same room	109 (27%)	105 (26%)
Monthly household income, 1000 Nepalese Rupees‡	30 (20–50)	30 (20–50)
Family receive remittance from abroad	43 (11%)	42 (11%)
Haemoglobin and biomarker concentrations		
Haemoglobin concentration, g/dL§	12·4 (1·1)	12·4 (1·1)
Haemoglobin concentration <11·3 g/dL¶	56/386 (15%)	54/384 (14%)
Vitamin B12 concentration, pmol/L	194 (80)	187 (72)
Vitamin B12 concentration <148 pmol/L	126/397 (32%)	137/399 (34%)
Vitamin B12 concentration <221 pmol/L	279/397 (70%)	290/399 (73%)
Total homocysteine concentration, µmol/L‖	6·1 (1·4)	6·1 (1·3)
Methylmalonic acid concentration, µmol/L‖	0·20 (1·90)	0·21 (1·79)
Folate concentration, nmol/L	64·1 (52·3)	69·8 (74·6)
3CB12**	0·07 (0·63)	0·03 (0·59)

Data are n (%), mean (SD), or median (IQR), or geometric mean (geometric mean SD factor). *Gestational week was calculated based on last menstruation period (two mothers in the vitamin B12 group and two mothers in the placebo group did not know last menstruation period). †Salaried jobs defined as jobs with regular monthly payment including government or private sectors. ‡1 Nepalese Rupee=US$0·008 (as of June, 2022); no information on household incomes from five families (two families in the vitamin B12 group and three families in the placebo group), §Data missing for 30 pregnant mothers (14 in the vitamin B12 group and 16 in the placebo group) due to unavailability of Hemocue cuvettes. ¶Indicative of anaemia, altitude adjusted. ‖Data are geometric mean and geometric mean SD factor, which is the exponential of the SD of the mean of the log-transformed values. **Combined indicator of vitamin B12 status based on vitamin B12 concentration, total homocysteine concentration, and methylmalonic acid concentration.

Table 1: **Maternal and household baseline characteristics according to intervention group**

Procedures

The supplements were produced specifically for this trial (GCRieber, Bergen, Norway) and provided in daily rations of solid 7 g biscuits (resembling local sweets) wrapped in foil from early pregnancy to 6 months post partum. During enrolment procedures, a research assistant provided the first week supply of biscuits to the participants with detailed information concerning dose and storage. In home visits that took place once a week, the participants were provided with refills. Participants in both study groups also received daily 400 µg of folic acid during the first trimester, and 60 mg of elemental iron and 0·5–1·0 g of calcium (once per day in pill form) from the second trimester until 45 days post partum according to national recommendations.

Details of antenatal supplementation, demographics, and socioeconomic characteristics were collected within 1 week of enrolment. Assessment procedures, response categories, and source of information are described in the appendix (pp 3–4). During the weekly visits to the participants' home in the follow-up period, we documented day-to-day adherence to the study intervention, intake of different foods, morbidities and symptoms, and hospital visits (appendix pp 5–7). During the COVID-19 pandemic and strict nationwide lockdowns (from March to July, 2020, and from April to June, 2021), this information was collected over the telephone. Frequency of antenatal visits, laboratory investigations, and other treatments were done as per the recommendations of the treating gynaecologist. According to study procedures, planned assessments and blood draw were conducted at the study clinic at 8 months of gestation and when the child was aged 8–12 weeks, 6 months, and 12 months.

Outcomes

The primary outcomes of the trial were linear growth expressed as length-for-age Z score (LAZ) at age 12 months and neurodevelopment as measured by the cognitive composite score of the Bayley-III[23] at age 6 months and 12 months. We used generalised estimating equations (GEE) models to take repeated measurements for individual an child into account. Key secondary infant outcomes were LAZ at age 6 months; weight-for-age Z score (WAZ), weight-for-length Z score (WLZ), BMI Z score, infant weight (kg), and infant length (cm) at age 6 months and 12 months; Bayley-III language, motor, and socioemotional composite scores at age 6 months and 12 months; and early motor performance measured by the Test of Infant Motor Performance (TIMP)[24] at age 8–12 weeks. Key secondary pregnancy and perinatal outcomes included congenital anomalies, gestational length, birthweight, and haemoglobin concentration.

Length and birthweight were measured at the study hospital and in the homes. The primary growth outcome was measured at the study hospital. Length was measured to the nearest millimetre using an infantometer (Seca, Hamburg, Germany). An electronic

scale was used to measure weight to the nearest 100 g (Seca). During training, the 18 fieldworkers reached an intra-class correlation coefficient (ICC) of 0·93–0·98 compared with an expert. During the implementation of the study, each child was measured twice, independently by two fieldworkers. The mean of the two measurements was used for the analyses. The technical error of measurement during the study was 0·18 cm for length and 0·03 kg for weight. LAZ, WAZ, and WLZ were calculated using WHO Child Growth Standards.

Bayley-III assessments were done with the child at the study clinic in rooms that were well lit and free from distractions. A caregiver was present with the child during testing, and children were well fed and not sick before the assessment. Three psychologists who had experience with Bayley assessments were standard in the assessment procedures, reaching an ICC above 0·85 when compared with a gold standard ahead of the study and an ICC above 0·94 for the double scoring during the study. Raw scores were converted to three composite scores: the cognitive, language, and motor composite score (expected SD of 15) on the basis of norms developed in the USA.[23] The assessments also included the questionnaire-based Bayley-III socioemotional scale.

The TIMP assessment was done at the study clinic by two trained examiners, reaching an ICC above 0·94 from the standardisation procedures ahead of the study and an ICC above 0·93 from double scoring during the study. The total possible score for the TIMP is 142, with higher scores indicating better motor performance and lower scores indicating worse motor performance. The TIMP has age-specific norms developed in the USA, categorising the motor performance into average, low-average, below-average, and far below-average range.

Assessment procedures for the pregnancy and perinatal outcomes and for the adverse events including miscarriage, number of medical terminations of pregnancy, stillbirth, admission to hospital of the mother, and infant deaths are described in the appendix (pp 3–4).

Laboratory procedures

Venous blood samples were collected from the mother at enrolment, 8 months of gestation, and 6 months after delivery, and from infants at age 6 months and 12 months. Approximately 3–4 mL of blood was collected into vials containing EDTA. The haemoglobin concentration was estimated immediately with the HemoCue Hb 201 System (HemoCue, Ångelholm, Sweden), calibrated as per guidelines set by the manufacturer. The plasma vials were centrifuged at approximately 2000 g at room temperature for 10 min, were stored at –70°C at the field site, and were always transported on dry ice.

Plasma concentrations of vitamin B12 were measured by microbiological assays using a colistinsulfate-resistant strain of *Lactobacillus leichmannii* and plasma concentrations of folate were measured by microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus casei*. The functional biomarkers plasma total homocysteine and methylmalonic acid were analysed by gas chromatography tandem mass spectrometry based on methylchloroformate derivatisation. The within-day coefficient of variability was 4% for both folate and vitamin B12 and ranged from 1% to 5% for total homocysteine and methylmalonic acid. The between-day coefficient of variability was 5% for both folate and vitamin B12 and ranged from 1% to 3% for total homocysteine and methylmalonic acid. These

	Vitamin B12	Placebo	Difference (95% CI)
Primary outcomes			
Anthropometry			
N	366	363	..
LAZ at age 12 months	–0·57 (1·03)	–0·55 (1·03)	–0·02 (–0·16 to 0·13)
Neurodevelopment			
N	727*	731*	..
Bayley-III cognitive composite score at age 6 and 12 months†	97·7 (10·5)	97·1 (10·2)	0·5 (–0·6 to 1·7)
Key secondary outcomes			
Anthropometry at age 6 months			
N	363	370	..
LAZ	–0·5 (1·0)	–0·4 (1·0)	–0·1 (–0·2 to 0·1)
Length, cm	65·6 (2·3)	66·0 (2·3)	–0·3 (–0·6 to 0·02)
WAZ	–0·20 (1·10)	–0·13 (1·10)	–0·07 (–0·23 to 0·09)
WLZ	0·22 (1·07)	0·25 (1·06)	–0·03 (–0·19 to 0·12)
Weight, kg	7·5 (1·0)	7·6 (1·0)	–0·1 (–0·3 to 0·03)
BMI z-score	0·11 (1·09)	0·15 (1·08)	0·04 (–0·20 to 0·12)
Anthropometry at age 12 months			
N	366	363	..
Length, cm	73·6 (2·7)	73·8 (2·7)	–0·2 (–0·6 to 0·2)
WAZ	–0·32 (1·06)	–0·26 (1·04)	–0·07 (–0·22 to 0·09)
WLZ	–0·07 (1·07)	0·01 (1·03)	–0·08 (–0·23 to 0·08)
Weight, kg	9·0 (1·2)	9·2 (1·2)	–0·1 (–0·3 to 0·04)
BMI z-score	0·01 (1·06)	0·09 (1·02)	–0·08 (–0·23 to 0·07)
Neurodevelopment (Bayley-III scores) at age 6 months			
N	363	370	..
Cognitive composite score	100·1 (10·1)	98·9 (10·7)	1·1 (–0·4 to 2·6)
Language composite score	88·3 (8·0)	88·9 (7·5)	–0·5 (–1·6 to 0·6)
Motor composite score	99·8 (13·1)	99·6 (13·7)	0·2 (–1·8 to 2·1)
Socioemotional composite score	100·6 (17·5)	101·2 (16·6)	–0·6 (–3·1 to 1·8)
Neurodevelopment (Bayley-III scores) at age 12 months			
N	364	361	..
Cognitive composite score	95·4 (10·4)	95·3 (9·2)	0·03 (–1·4 to 1·5)
Language composite score	77·2 (12·2)	76·7 (11·5)	0·5 (–1·2 to 2·5)
Motor composite score	93·8 (11·1)	94·0 (12·0)	–0·2 (–1·9 to 1·5)
Socioemotional composite score	100·1 (16·9)	100·3 (15·9)	–0·3 (–2·7 to 2·1)
TIMP at age 8–12 weeks			
N	357	355	..
TIMP total score	76·2 (10·4)	78·0 (9·1)	–1·8 (–3·3 to –0·4)
TIMP score below average range	181 (51%)	136 (38%)	12·4 (5·1 to 19·6)

(Table 2 continues on next page)

	Vitamin B12	Placebo	Difference (95% CI)
(Continued from previous page)			
Haemoglobin and biomarker concentrations of infant at age 6 months			
N	375	377	..
Haemoglobin concentration, g/dL	11·2 (1·1)	11·3 (1·1)	−0·1 (−0·3 to 0·1)
Haemoglobin concentration <11·3 g/dL‡	199 (53%)	188 (50%)	3·1 (−3·4 to 10·3)
N	127	129	
Vitamin B12 concentration, pmol/L§	222 (79)	192 (60)	31 (13 to 48)
Total homocysteine concentration, µmol/L	7·3 (1·3)	10·8 (1·5)	1·5 (1·4 to 1·6)§
Methylmalonic acid concentration, µmol/L	0·25 (1·84)	0·42 (2·10)	1·7 (1·4 to 2·0)§
Folate concentration, nmol/L	51·3 (21·5)	66·1 (25·8)	−14·8 (−20·6 to −8·9)
3CB12¶	−0·2 (0·6)	−0·8 (0·7)	0·6 (0·5 to 0·8)
Haemoglobin and biomarker concentrations of infant at age 12 months (6 months after end of supplementation)			
N	362	363	..
Haemoglobin concentration, g/dL	11·1 (1·2)	11·2 (1·2)	−0·1 (−0·2 to 0·1)
Haemoglobin concentration <11·3 g/dL‡	202 (56%)	186 (51%)	4·6 (−2·7 to 11·8)
N	134	131	
Vitamin B12 concentration (pmol/L) §	225 (90)	217 (92)	8 (−15 to 30)
Total homocysteine concentration, µmol/L	6·9 (1·3)	8·4 (1·4)	1·2 (1·1 to 1·3)§
Methylmalonic acid concentration, µmol/L	0·32 (2·1)	0·43 (2·1)	1·4 (1·1 to 1·6)§
Folate concentration, nmol/L	47·8 (20·1)	49·7 (20·2)	−1·9 (−6·8 to 2·0)
3CB12¶	−0·3 (0·7)	−0·6 (0·7)	0·3 (0·2 to 0·5)

Data are N, mean (SD), or n (%). Numbers of infants differ between the outcomes because it was not always possible to measure the outcome when it was due (eg, because the infant was not available or not able to cooperate). Bayley-III=Bayley Scales of Infant and Toddler Development, 3rd edition. LAZ=length-for-age Z score. TIMP=Test of Infant Motor Performance. WAZ=weight-for-age Z score. WLZ=weight-for-length Z score. *Total number of infants in each group at two timepoints (age 6 months and 12 months). †Generalised estimating equation with two timepoints (age 6 and 12 months). ‡Indicative of anaemia, altitude adjusted. §Data are geometric mean and geometric mean SD factor, which is the exponential of the SD of the mean of the log-transformed values; an effect size of 1·5 indicates that the concentration is 50% higher in the placebo group than in the vitamin B12 group. ¶Combined indicator of vitamin B12 status based on vitamin B12 concentration, total homocysteine concentration, and methylmalonic acid concentration.

Table 2: **Primary and secondary outcomes in infants**

For the **Bevital Laboratory** see www.bevital.no

biomarkers were analysed at Bevital Laboratory, Bergen, Norway. We also calculated the combined indicator of vitamin B12 status (3CB12) on the basis of vitamin B12 concentration, total homocysteine concentration, and methylmalonic acid concentration.[25] Higher 3CB12 indicates better status.

Statistical analysis

The analyses were carried out according to a predefined protocol and statistical analysis plan that was uploaded to ClinicalTrials.gov before the statistical analyses commenced. The primary and secondary outcomes were assessed in the intention-to-treat population. Before the trial groups were unblinded, we completed data cleaning and curation for all the variables included in the analyses. We used generalised linear models (GLMs) with the Gaussian distribution family and identity link function for the primary growth outcome and most of the secondary outcomes. Supplementation status was the only independent variable in these GLM models. For the primary outcome cognitive composite scores, where measurements both at age 6 months and 12 months were used simultaneously, we used GEE models with an exchangeable covariance structure to take the repeated measurements into account.[26] We compared proportions using GLM with the binomial distribution family and identity link function, yielding differences in proportions (or risks). We log-transformed plasma total homocysteine and methylmalonic acid concentrations because these data were left-skewed, and present the exponentials of their means, SDs, and mean differences as geometric means, geometric SD factors, and geometric mean ratios. The intervention response in vitamin B12 status in mothers and children was visualised using Epanechnikov kernel density plots of the plasma vitamin B12 and log-transformed total homocysteine and methylmalonic acid concentrations. We also present the effects of the intervention separately in those with the highest and lowest baseline vitamin B12 status (assessed using 3cB12). Because the 3CB12 has not been validated for pregnant women, we categorised the 3CB12 at the 33rd percentile. Adverse events during the supplementation period are presented as the number of days or number of events with episodes of symptoms, admission to hospital of the mother, and visits to health facilities by study group. The sample size was established using the power function in Stata (College Station, TX, USA). For a standardised effect size of 0·25 SD, 676 mothers were required to have a statistical power of 90% (α=5%). We did not adjust the α for multiple comparisons (more than one main outcome). With a potential loss to follow-up of 15% (mothers or children) due to dropout or miscarriage,[27] the final sample size was set to 800 women. The trial was registered with ClinicalTrials.gov, NCT03071666.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From March 28, 2017, to Oct 15, 2020, we screened 1394 pregnant women for eligibility, and 800 were enrolled in the trial (figure 1). Follow-up for the primary outcomes was completed on May 18, 2022. A total of 47 (6%) of 800 participants were lost to follow-up, mainly due to spontaneous abortion or medical termination after detection of severe anomaly by ultrasonography. There were 760 livebirths, 361 (47%) of which were caesarean sections. 755 (94%) children were

still in the study at follow-up at age 6 months and 753 (94%) children were still in the study at follow-up at age 12 months. We were unable to assess 22 (3%) children at age 6 months and 28 (4%) children at age 12 months with the Bayley-III within the predefined window period (within 45 days) mainly due to the lockdowns during the COVID-19 pandemic or due to illness.

Baseline characteristics are described in table 1. The mean maternal age was 27·7 (SD 4·0) years, 390 (49%) pregnant women were primipara and 464 (58%) pregnant women were enrolled within the first trimester of pregnancy. According to the plasma vitamin B12 cutoff lower than 221 pmol/L, 279 (70%) of 397 mothrs in the vitamin B12 group and 290 (73%) of 399 mothers in the placebo group had low or marginal vitamin B12 status at enrolment. For all participants, we documented supplement adherence for a total of 276 435 days. Of these days, the supplement was not consumed for 5902 (4%) of 137 392 days in the vitamin B12 group and for 6061 (4%) of 139 043 days in the placebo group. Reasons for not taking supplements were mainly unscheduled travel out of the study area during COVID-19 lockdowns or admission to hospital of the mother for childbirth (appendix p 5). The infant and maternal vitamin B12 biomarker concentrations show a substantial metabolic response to the vitamin B12 supplementation (tables 2, 3; appendix p 8). The responses over the whole distribution of the biomarkers both in mothers and infants are shown in figure 2. The frequency of intake of various food items are shown in the appendix (p 7).

At the visit at age 12 months, the mean LAZ of infants was –0·57 (SD 1·03) in the vitamin B12 group (366 infants) and –0·55 (1·03) in the placebo group (363 infants), with a mean difference between study groups of –0·02 (95% CI –0·16 to 0·13). The mean length of infants at age 12 months in the vitamin B12 group was 73·6 cm (SD 2·7) and the mean length of infants in the placebo group was 73·8 cm (2·7), whereas the mean weight of infants in the vitamin B12 group was 9·0 kg (1·2) and the mean weight of infants in the placebo group was 9·2 kg (1·2; table 2). There were no between-group differences for any other anthropometric index (table 2).

There was no difference between the groups in any of the Bayley-III scores at any timepoint. The mean Bayley-III cognitive composite score (primary outcome) in the intervention group was 97·7 (SD 10·5) and the mean score in the placebo group was 97·1 (10·2). The mean difference from the GEE model was 0·5 (95% CI –0·6 to 1·7).

There was no effect of vitamin B12 supplementation on birthweight (mean of 2990·2 (SD 456·0) g in the vitamin B12 group and 3048·1 (446·8) g in the placebo group). Similarly, there was no effect of vitamin B12 supplementation on the proportion of infants born with low birthweight or born preterm (table 3).

	Vitamin B12	Placebo	Difference (95% CI)
Pregnancy and birth outcomes			
N	377	383	..
Gestational length, weeks	38·4 (1·8)	38·5 (1·9)	–0·1 (–0·3 to 0·3)
Preterm delivery (<37 weeks)	37 (10%)	33 (9%)	1·3 (–2·8 to 5·4)
Congenital anomaly*	12 (3%)	6 (2%)	–1·6 (–3·8 to 0·5)
Birthweight, g	2990·2 (456·0)	3048·1 (446·8)	–57·0 (–121·8 to 7·8)
<2500 g	39 (10%)	32 (8%)	–1·8 (–6·0 to 2·4)
<2000 g	9 (2%)	5 (1%)	–1·1 (–3·1 to 0·9
>4000 g	7 (2%)	3 (1%)	–0·8 (–2·4 to 0·7)
Haemoglobin and biomarker concentrations of mother at 8 months during pregnancy			
N	186	184	..
Haemoglobin concentration, g/dL	11·6 (1·3)	11·6 (1·2)	0·1 (–0·1 to 0·3)
Vitamin B12 concentration, pmol/L	243 (72)	160 (51)	83 (70 to 95)
Vitamin B12 <148 pmol/L	11 (6%)	85 (46%)	–40·3 (–48·2 to –32·3)
Total homocysteine concentration, µmol/L geometric mean†	5·6 (14)	6·5 (1·4)	1·2 (1·0 to 1·8)†
Methylmalonic acid concentration, µmol/L geometric mean†	0·19 (1·62)	0·32 (1·70)	1·7 (1·6 to 1·9)†
Folate concentration (nmol/L)	20·3 (13·9)	21·3 (12·5)	–1·0 (–3·7 to 1·8)
3CB12‡	0·3 (0·5)	–0·3 (0·6)	0·7 (0·6 to 0·8)
Haemoglobin and biomarker concentrations of mother at 6 months postpartum			
N	127	129	..
Haemoglobin concentration, g/dL	13·2 (1·1)	13·3 (1·0)	-0·1 (-0·3 to 0·1)
Vitamin B12 concentration, pmol/L	268 (102)	182 (49)	86 (67 to 106)
Vitamin B12 concentration <148 pmol/L	9 (7%)	31 (24%)	–16·9 (–25·5 to –0·1)
Total homocysteine concentration, µmol/L	6·8 (1·3)	8·5 (1·4)	1·2 (1·2 to 1·3)†
Methylmalonic acid concentration, µmol/L	0·17 (1·55)	0·27 (1·73)	1·6 (1·4 to 1·8)†
Folate concentration, nmol/L	18·7 (9·1)	17·7 (7·9)	1·0 (–1·0 to 3·1)
3CB12‡	0·3 (0·5)	–0·3 (0·5)	0·6 (0·5 to 0·8)

Data are N, mean (SD), or n (%). *The following congenital anomalies were identified during first visit after delivery: congenital heart disease, atrial septum defect, patent ductus arteriosus, cleft palate, polydactyly and others (eg, undescended testes, laryngomalacia, pes planus, hypoplastic phalanges). †Data are geometric mean and geometric mean SD factor, which is the exponential of the SD of the mean of the log-transformed values; an effect size of 1·2 indicates that the concentration is 20% higher in the placebo group than in the vitamin B12 group. ‡Combined indicator of vitamin B12 status based on vitamin B12 concentration, total homocysteine concentration, and methylmalonic acid concentration.

Table 3: **Pregnancy and birth outcomes, and maternal biochemical response during pregnancy and 6 months postpartum**

Maternal vitamin B12 supplementation resulted in 1·8 (0·4–3·3) lower TIMP total score (age 8–12 weeks) when compared with placebo, and a 12·4% (percentage points) higher risk of having scores below the average range (95% CI 5·1–19·6), based on the norms developed in the USA (table 2). TIMP score was a predefined secondary outcome of the study. As shown by an a priori subgroup analysis, the negative effect of vitamin B12 supplementation on the TIMP score was stronger when restricting the analysis to the 531 (66%) women with the best baseline vitamin B12 status (appendix pp 9–11).

Subgroup analyses according to vitamin B12 status of pregnant women at enrolment did not reveal any other

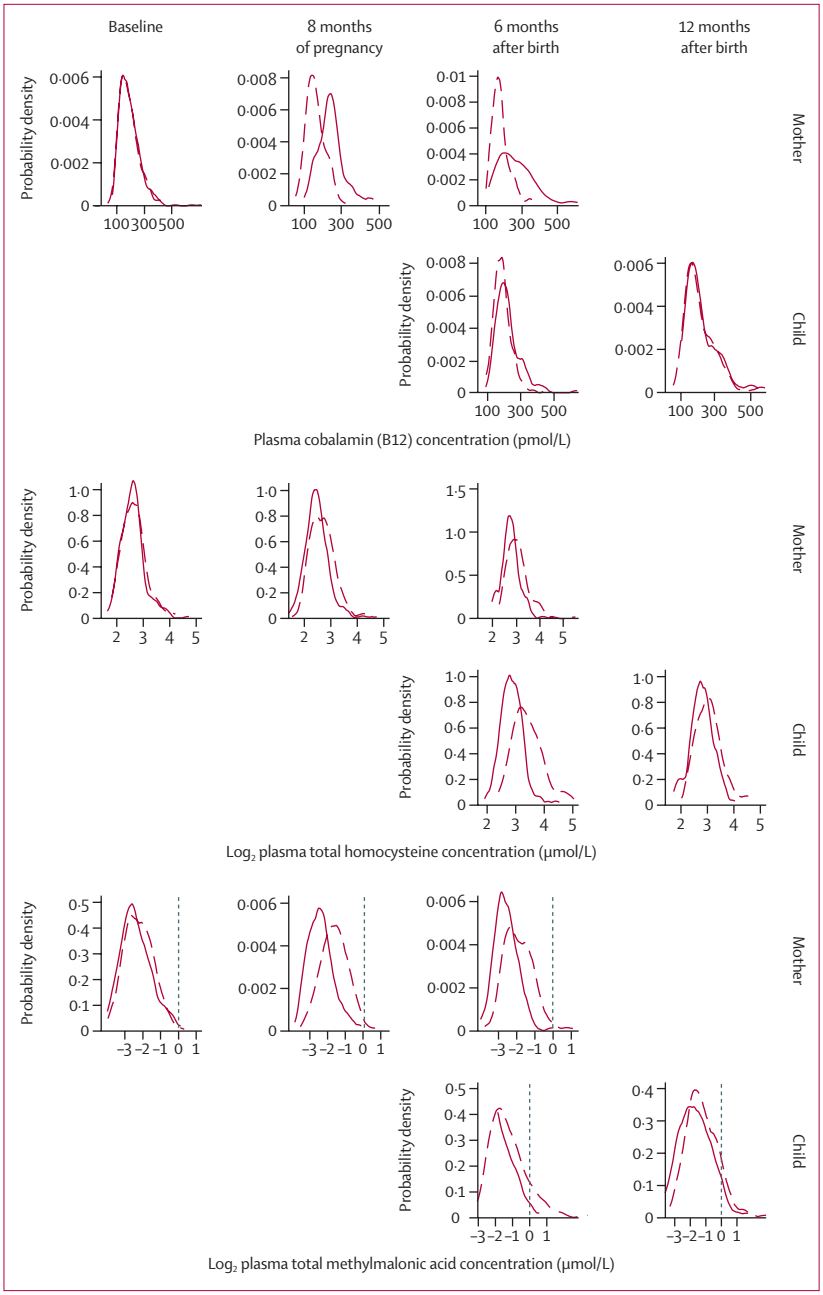

Figure 2: **The effect of vitamin B12 supplementation during pregnancy and post partum on the distribution of plasma vitamin B12, total homocysteine, and methylmalonic acid in mothers and infants at different timepoints**

Distribution of plasma vitamin B12 and log-transferred total homocysteine and methylmalonic acid concentrations at four timepoints (mothers: baseline, 8th month of pregnancy, and 6 months after delivery; infants: 6 and 12 months of age) by treatment group. Solid lines represent the vitamin B12 group and dashed lines represent the placebo group.

effects of vitamin B12 supplementation on primary or secondary outcomes (appendix pp 9–11). We observed few adverse events before age 12 months: one (<1%) stillbirth occurred in the vitamin B12 group and five (1%) stillbirths occurred in the placebo group. Two (1%) infant deaths occurred in the vitamin B12 group and four (1%) infant deaths occurred in the placebo group (table 4).

	Vitamin B12 (n=400)	Placebo (n=400)
Miscarriage	11 (28%)	4 (1%)
Medical termination*	8 (2%)	4 (1%)
Stillbirth	1 (<1%)	5 (1%)
Admission to hospital of the mother†	68 (17%)	49 (12%)
Infant death (age 0–12 months)	2 (1%)	4 (1%)
Due to neonatal sepsis	2 (1%)	2 (1%)
Due to extreme prematurity	0	1 (<1%)
Due to complex congenital heart disease	0	1 (<1%)

*Due to severe anomaly detected during ultrasonography (eg, holoprosencephaly, cystic hygroma, hydrops fetalis, anencephaly, or gross hydrocephalus). †Mainly due to threatened abortion, urinary tract infection, oligohydramnios, hypertension, hyperemesis gravidarum, eclampsia or pre-eclampsia, and decreased fetal heart sound.

Table 4: **Severe adverse pregnancy and infant outcomes**

Discussion

This study was conducted in an area with low vitamin B12 intake, where low and marginal vitamin B12 status is endemic,[19] and where we have previously shown associations between maternal vitamin B12 status, child growth, and neurodevelopment.[21] Vitamin B12 supplementation (50 μg vitamin B12 per day) from early pregnancy until 6 months post partum did not improve infant growth and neurodevelopment despite a substantial biomarker response and improved B12 status among mothers and infants.

The absence of an effect of vitamin B12 supplementation on our primary outcomes contrasts with previous studies that suggest that even marginal vitamin B12 status contributes to impaired growth[9,21] and neurodevelopment.[8,10] However, our findings are supported by results from a previous RCT conducted in the same area, in which 1 year of daily B12 supplementation starting from infancy had no effect on infant growth and neurodevelopment.[20] Two RCTs published between 2017 and 2021 from India, which involved vitamin B12 supplementation during pregnancy, found positive effects on specific neurodevelopmental domains.[11–13] However, these studies were small and neurodevelopment was a secondary outcome, which contrasts with our well powered study that assessed neurodevelopment as the primary outcome.

We did not identify beneficial effects of vitamin B12 supplementation on any of the secondary outcomes or when stratifying the analyses according to maternal vitamin B12 status at enrolment. On the contrary, we found a negative effect of vitamin B12 supplementation on motor performance (assessed by total TIMP score) at age 8–12 weeks. This finding raises the concern that there

might be possible adverse effects of supplementing vitamin B12 during pregnancy. It should be noted that there was no effect on motor scores of the Bayley-III at age 6 or 12 months, suggesting that either the negative effect on motor performance did not persist, or that the adverse effect on infant motor performance at age 8–12 weeks was a spurious finding. However, despite being a secondary outcome, the adverse effect on early motor performance gives reason for caution when supplementing with B12 during pregnancy. It should be noted that the clinical significance of the observed difference in a test of motor function at this age is uncertain and these results should be interpreted with caution.

The comprehensive characterisation of biomarkers related to vitamin B12 status in both women and infants at several timepoints are an important reassurance on both the dose sufficiency and intervention adherence. We observed a high prevalence (71%) of low and marginal vitamin B12 status in mothers at enrolment. We also observed a favourable metabolic response to vitamin B12 supplementation in both mothers and infants (figure 2), with a substantial shift in the distribution of different biomarker concentrations between the placebo and intervention group. At the end of pregnancy, 11 (6%) of 186 women in the vitamin B12 group had vitamin B12 deficiency (vitamin B12 concentration <148 pmol/L), and 85 (46%) of 186 women in the placebo group were deficient (vitamin B12 concentration <148 pmol/L). Beyond documenting adherence to B12 supplementation, this observed shift indicates a biochemical effect of supplementation that could have consequences on clinical outcomes (eg, long-term metabolic health) that are beyond the scope of this report.

Our null results could have several explanations. It is possible that most of the women did not have enough vitamin B12 deficiency in the vitamin B12 group (eg, overt clinical signs of vitamin B12 deficiency was an exclusion criterion) or that the supplementation period did not cover a crucial window relevant to our outcomes. Thus, initiating supplementation sooner (eg, before pregnancy) could have yielded different results. We could also have seen effects with a higher dose of vitamin B12 than the one we used; however, despite scarce evidence, we cannot exclude that a higher dose might have increased the risk of negative consequences. It is possible that some women and children might have benefited from supplementation. The effect in these women and children, however, was not measurable because it only contributed to the population average among many who had no or even a negative effect. In other words, the positive effect of the few was diluted by the response in most of the study participants. Studies designed to precisely identify those likely to benefit from vitamin B12 supplementation are warranted. These studies should target populations with higher amounts of vitamin B12 deficiency.

Although vitamin B12 supplementation has been suggested as an intervention among populations in which low and marginal vitamin B12 status is common,[16,17] our study adds to the current literature that evidence is not sufficient to recommend B12 supplementation during pregnancy as a routine preventive measure. However, our biomarker results substantiate poor vitamin B12 status among pregnant women in the Bhaktapur municipality and surrounding areas in Nepal. Notably, using a plasma vitamin B12 cutoff of 250 pmol/L as suggested by others,[28] 653 (82%) of 796 women had subclinical deficiency of vitamin B12. The positive predictive value of diagnostic tests can be substantially attenuated when used in non-clinical settings where most of the population is without overt signs and symptoms of deficiency, such as in this study. Caution should therefore be used when using biochemical indices to advocate fortification and supplementation. We believe that our findings can be generalised to other populations in which deficient vitamin B12 status is prevalent, which could include populations both in high-income and low-income settings.

The strengths of the study include the large sample size, successful randomisation, close follow-up, few losses to follow-up, and excellent adherence to the study intervention. Further strengths are the high-quality assessments of outcomes, including the high inter-rater agreement on the neurodevelopmental outcomes, and that neurodevelopment was measured at two timepoints. The many secondary outcomes included in our study are also an asset, because they reduced the risk of important clinical effects of vitamin B12 supplementation being overlooked. Furthermore, the total homocysteine and methylmalonic acid response to the supplementation support excellent adherence and further indicate the deficient vitamin B12 status in the target population. The results of this study should be interpreted considering some limitations. Neurodevelopment assessments show low stability in early childhood;[29,30] thus, assessments later in childhood (eg, during the first years of school) could give more reliable and detailed information on the effect of the intervention. In addition, due to lockdowns during the COVID-19 pandemic, we could not assess growth and neurodevelopment within the window period in all children. However, the number of children excluded from the analysis was low.

In summary, there was no beneficial effect of vitamin B12 supplementation from early pregnancy until 6 months post partum on infant growth, infant neurodevelopment, and pregnancy outcomes, but we observed a possible negative effect on infant motor performance at age 8–12 weeks that should be interpreted cautiously. Findings from the study support the current WHO recommendations of no routine vitamin B12 supplementation during pregnancy.[15]

Contributors

TAS was the principal investigator for the trial and conceived the study, and supervised and performed the statistical analyses, interpretation of results, and drafting of the manuscript. RKC and LS were the local

principal investigators for the trial and contributed to design, data collection, supervision, data analyses, and drafting of the manuscript. IK and MH contributed to study design, supervised the cognitive assessment, and contributed to the statistical analysis and drafting of the manuscript. MU, SR, MS, and SB contributed to study design, study implementation, logistic support, supervision, and drafting the manuscript. CS contributed to the statistical analysis, literature search, and drafting of the manuscript. AM contributed to laboratory analyses, interpretation of results, and drafting and review of the manuscript. PMU contributed to laboratory analyses and critical review of the manuscript. TAS had full access to all the data in the study and RKC, IK, and CS accessed and verified the data. All authors had permission to access the raw data. All authors shared final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Data is available on request. To meet ethical requirements for the use of confidential patient data, requests must be approved by the Nepal Health Research Council (NHRC) and the Regional Committee for Medical and Health Research Ethics in Norway. Requests for data should be sent to the authors, by contacting NHRC (http://nhrc.gov.np), or by contacting the Department of Global Health and Primary Care at the University of Bergen (post@igs.uib.no).

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