The association between socioeconomic status and linear growth in Nepalese children under 2 years of age

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To my son (Saarav)!

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Sweta Pathak (Bergen, May 2021)

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Acronyms and Abbreviations

DAG	Directed acyclic graph
IQR	Interquartile range
MAL-ED	Etiology, risk factors and interactions of enteric infections and malnutrition and the consequences for child health and development
NDHS	Nepal Demographic and Health Survey
SDG	Sustainable Development Goals
SES	Socioeconomic status
UN	United Nations
UNDP	United Nations Development Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations Children's Fund
WAMI	Water and sanitation, assets, maternal education, income
WHO	World Health Organization

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1. Introduction

1.1. Child growth

The early years of life are crucial for optimal physical growth and mental development. This is the period when all organ system are developing most rapidly, and a strong foundation is made for health and wellbeing throughout life [1-3]. This is one of the reasons why children under 5 years of age have a special place in the global health agenda. The first 5 years of children are important, but special focus has been on the first 1,000 days, which is a crucial time period from conception to the 2nd birthday. World Health Organization (WHO) advocates the importance of the first 1,000 days for child growth [4]. During these first 1,000 days, good nutrition and health care makes a strong foundation for optimum health, growth, and neurodevelopment [4]. Therefore, it is important to make sure that young children grow up in favourable environments in order to achieve optimal growth and development.

Anthropometric measurements such as length/height, weight, and head circumference are used to assess and monitor child growth [5]. To evaluate child growth, anthropometric measurements are commonly compared with WHO standards [6]. Poor growth can be a sign of poor nutrition, poor health, or insufficient health care [7 8]. In other words, child growth is a proxy for child health. United Nations Children's Fund (UNICEF) has highlighted the importance of nutrition on child growth, especially in first 1,000 days of life in different health reports [9]. Lancet series (2008) reported that the child's linear growth failure was associated with inadequate diet and frequent infection during intrauterine period and first two years of life [10]. Furthermore, a meta-analysis observed that linear growth in the first 2 years of life was strongly associated with both early and later childhood cognition compared with growth after first 2 years of life [11].

Below is the background to the thesis, which includes an overview of the concepts of malnutrition, undernutrition, and stunting, anthropometric scoring, determinants and consequences of child growth, and socioeconomic status (SES) as a determinant of child growth.

1.2. Malnutrition

WHO defines malnutrition as deficiency, excess, or imbalances in nutrient intake. Deficiencies in food or nutrient intake can lead to undernutrition, whereas excess amount can lead to overnutrition or obesity [4]. A double burden of malnutrition exists when two or more forms of malnutrition are present at same time in a population, a household or an individual.

There is a growing double burden of malnutrition in low- and middle-income countries such as Nepal, where the problem of undernutrition persists, and the prevalence of obesity is increasing. This has received more attention of national and international authorities in the last years [12].

In this thesis, we focused on the undernutrition aspect of malnutrition.

1.3. Undernutrition

According to WHO, it is estimated that 5.3 million children under 5 years of age died across the globe in 2018, which equals to 15,000 children each day [13]. Nearly half of these deaths have undernutrition as an underlying factor, which puts children at greater risk of dying from common childhood illness such as pneumonia, diarrhoea, and malaria [4]. Undernutrition is common in low- and middle-income countries where approximately only 1 in 5 children between the 6 months and 5 years of age eat a sufficiently diverse and healthy diet [14]. Pregnant women who suffer from undernutrition are likely to give birth to children who will suffer from stunting or wasting. In this way, intergeneration cycle of undernutrition continues[15].

To overcome undernutrition among children under 5 years of age, many plans and policies have been formulated, different targets have been set and also a lot of activities have been carried out in the community, and at national and international level. Despite the fact that achievement has been made, according to a recent report published by UNICEF in 2020 it shows that the progress is too slow [16]. According to the 2018 Global Nutrition Report, undernutrition is associated with morbidity and mortality, especially in children under 5 years of age [17]. Therefore, undernutrition is a great barrier for achieving the Sustainable Development Goals (SDGs) such as Goal 2: Zero hunger, which has aimed to end all form of malnutrition and hunger especially in children by 2030 and Goal 3.2: End preventable deaths of newborns and children under 5 years of age [18 19].

Common indicators for undernutrition are deficiencies in vitamins and minerals, underweight (low weight-for-age), wasting (low weight-for-length/height), and stunting (low length/height-for-age) [4 20]. This thesis focuses on child linear growth or stunting aspect of undernutrition.

Stunting

Stunting is defined as low length/height-for-age. According to WHO Child Growth Standards, children are classified as stunted if their length/height-for-age is less than or equal to two standard deviations below the WHO reference median (z-score ≤ -2), where a z-score ≤ -2 to >-3 is defined as moderate stunting and z-score ≤ -3 is defined as severe stunting [21]. Stunting in early life has been associated with poor motor and cognitive development, as well as behavioural abnormality [22]. Long term consequences in health include shorter adult height, poor school achievement, low work productivity and wages in adulthood, and risk of obesity and cardiovascular disease [23-25]. Huey et al. estimated that 14% of childhood deaths were due to stunting, and a meta-analysis observed that the risk of mortality increased with severity of stunting. The risk was even higher when stunting, wasting, and underweight were present at same time [26 27]. The first 1,000 days are suggested as crucial time for intervention in order to prevent stunting [28]. Several studies have observed that children who are stunted in their first 1,000 days may never meet their full physical and intellectual capability [14]. However, Young Lives research of University of Oxford studied 12,000 children from Ethiopia, India, Peru and Vietnam since 2002 and found that child growth trajectories are not only determined during infancy, but children could catch up in their linear growth beyond infancy with proper nutritional intervention [29]. However, stunting followed by rapid weight gain during first 1,000 days was associated with obesity in later life resulting in an increased risk of stroke, hypertension, coronary heart disease, and type 2 diabetes [30].

According to the UNICEF/WHO/World Bank Joint Child Malnutrition estimate, the global prevalence of stunting among children under 5 years of age has declined from 199.5 million to 144 million between 2000 and 2019 [31]. However, in West and Central Africa, the number has increased from 22.4 million in 2000 to 29 million in 2019 indicating the urgent need for action for prevention and treatment. Despite the decrease in South Asia, it has the highest prevalence of stunting compared to other regions across the globe. With an estimated 56.9 million stunted children, this is an alarming situation [31]. Globally, 3.6% of children under 5 years of age (15.9 million) are living with both stunting and wasting, and 1.9% (8.2 million) are living with both stunting and overweight [4 17]. In Nepal, the Nepal Demographic and Health Survey (NDHS) in 2016 observed that among all forms of malnutrition in children under 5 years of age, stunting accounted for the highest number; 36% were stunted and 12% were severely stunted [32]. To achieve the SDGs by 2030, Nepal has set a challenging target of reducing stunting to 12% by 2025 and 1% by 2030 in children under 5 year of age[33].

1.4. Anthropometric scoring

The growth/anthropometric references that had been used since 1970 were not adequately representing early childhood growth. Therefore, to generate internationally representative reference values for early childhood growth among children under 5 years of age, the WHO Multicentre Growth Reference Study was conducted between 1997 to 2003 in Brazil, Ghana, India, Norway, Oman, and USA among 8,440 healthy breastfeed infant. In April 2006, WHO launched the international child growth standards based on height and weight that describe attained growth of healthy children under 5 years of age [21 34]. Attained growth refers to growth at a single time point explaining the dimension the child has attained up to that time point. Therefore, it is a cumulative measure of all the events in the past [35 36]. Indices used to assess child growth based on attained growth are length/height-for-age, weight-for-length/height, and weight-for-age.

For countries like Nepal, who do not have a national growth reference, the WHO child growth reference is very helpful to assess child growth [37].

1.5. Determinants and consequences of child growth

In 1990, UNICEF published a conceptual framework of the multifactorial determinants of maternal and child undernutrition, in which they have categorized determinants as basic, underlying and immediate cause [38]. This framework was also used in the 2008 Lancet series on Maternal and Child Undernutrition [39]. Later, this framework was adapted in the 2013 Lancet series by adding possible intervention at different levels of determinants and showing the determinants of optimal fetal and child growth and development (Figure 1) [40].

The framework (Figure 1) explains the determinants for and consequences of optimal child growth with possible action to achieve optimal child growth [40]. The social, economic, and political factors are suggested as basic determinants. These have an impact on food security, maternal and childcare, health service, and healthy environments which are labelled as underlying determinants. These determinants, in turn, influence nutrient intake and disease/illness, which are labelled as immediate determinants for child growth and development [40]. Similarly, interventions and programmes are also categorized according to the level of determinants. The framework has also highlighted long- and short-term effects of poor child growth and development on health and its related outcomes including mortality, morbidity, poor cognitive-motor and socioeconomic development, obesity, and non-communicable diseases. Some evidence supporting this framework are described below [40].

A cohort study done in Ghana observed that risk of death increased exponentially with decreasing birth weight [41]. Other studies observed that children born with low birth weight are more likely to have intellectual and developmental disabilities and chronic condition like obesity, high blood pressure and diabetes [42 43]. Similarly, children with wasting have higher risk of developing chronic disease in later life [44-46]. Furthermore, it has been observed that children with wasting are 12 times more likely to die than well-nourished children [44-46]. The consequences of stunting are described in detail above (section 1.3). Therefore, all the determinants should be carefully and detailly taken into consideration with possible plans, policies, or interventions in order to deal with poor childhood growth and its consequences.

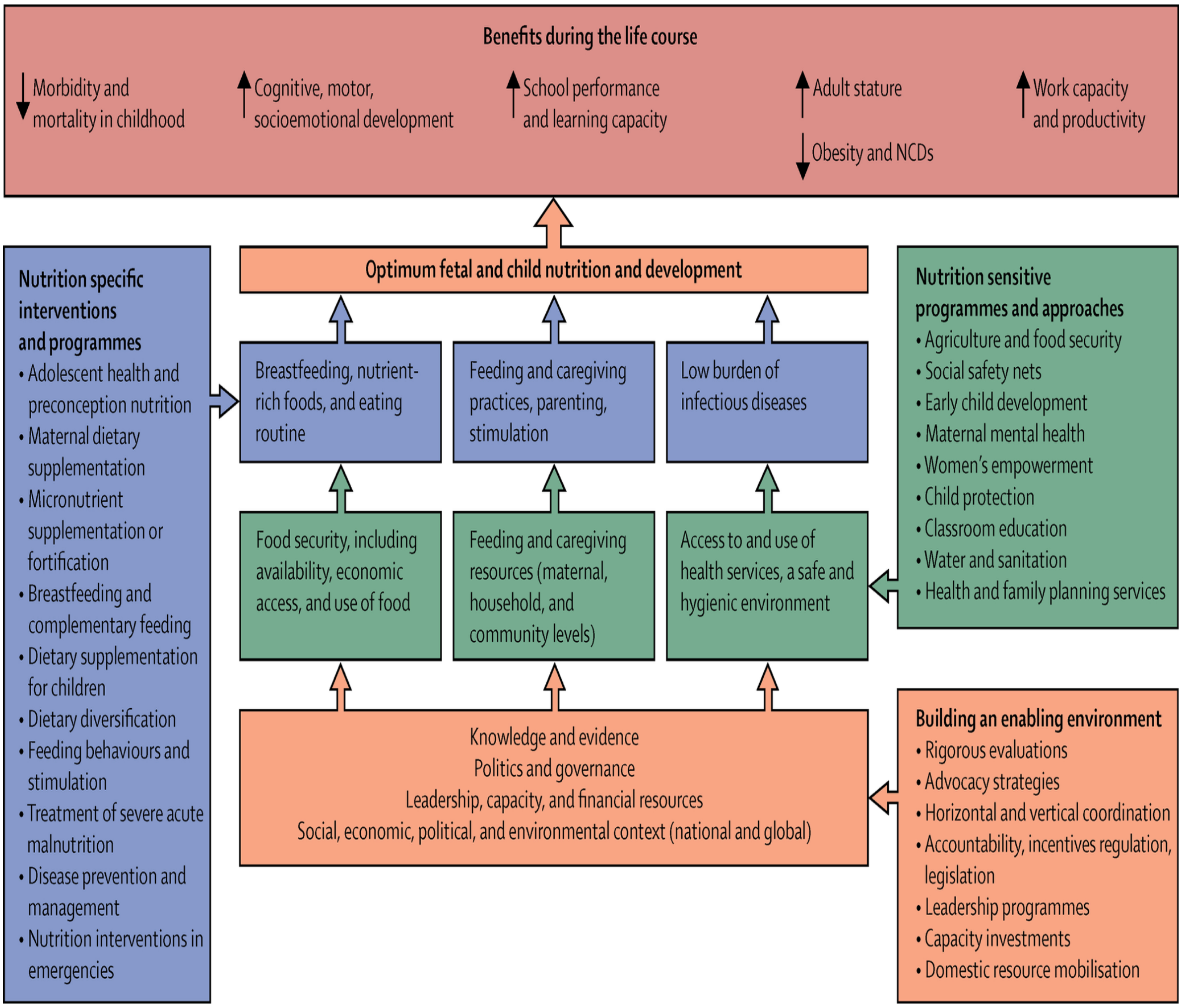

Figure 1. Framework for actions to achieve optimum fetal and child growth and development. The figure is reproduced from 2013 Lancet Series with permission from Elsevier.

WHO has highlighted the poor nutrition and repeated infections as the most immediate causes of stunting [47]. Maternal health and nutrition during pregnancy have an impact on the child linear growth. After the birth of the child, inadequate feeding practices and infection contribute to stunted growth [22].

SES is a determinant of child growth at the basic level, which determines the level of health care, food and nutrition, and disease and infection. This thesis focused specifically on SES as a determinant of child linear growth.

1.6. Socioeconomic status (SES)

The prevalence of undernutrition in childhood is contextual, and socio-economic status has been found to play an important role in growth of children [48-51]. A study by WHO observed socioeconomic inequalities in wasting and stunting in developing countries where the socioeconomic inequalities were more pronounced for stunting compared to wasting [51]. SES is commonly conceptualized as a combination of economic, social, and work status measured by income or wealth, education, and occupation, respectively [52]. The literature suggests a wide range of measures of SES and multiple ways to measure these. Different studies have used different measures of SES over the years [53]. A recent review of 256 articles included a wide range of measures of SES and observed that among all measures of SES, an asset-based wealth index was the most frequently used measure, followed by income and expenditure, occupation, education, and wealth index combined with education [54]. Mostly, studies have used single component measures of SES such as income, education, or occupation rather than multidimensional measures of SES [54]. The multidimensional measures of SES are suggested to best reflect the SES in a population and could have the potential to be generalized to other contexts [55]. In 2014, the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study conducted in 8 low- and middle-income countries representing rural, peri-urban, urban areas has introduced a multidimensional measure of SES termed as WAMI index [55]. The WAMI index accounts for access to improved water and sanitation (W), wealth measured by a set of assets (A), maternal education (M), and monthly household income (I). This was found to be strongly associated with stunting across the 8 countries [55]. The WAMI index is relatively simple to use and could provide a standardized approach to generalize SES across diverse population [55].

2. Rationale for the study

The first 1,000 days of life from conception to the 2nd birthday is considered as a crucial time period determining later life health outcomes [1-4]. Good nutrition and care during this period are an important contributor to the child's ability to grow and learn [9 10]. WHO and UNICEF have underlined the importance of child linear growth and also highlighted the effect of impaired linear growth or stunting in early childhood, especially in the first 1,000 days [47 56]. Amongst others, impaired linear growth is associated with poor cognitive development, poor educational performance, reduced intellectual capacity, low adult wages, and poor motor and socioeconomic development in later life [22-25 57]. Stunting followed by rapid weight gain during first 1,000 days has been found to be associated with obesity in later life resulting in an increased risk of stroke, hypertension, coronary heart disease and type 2 diabetes [30 47 56 58]. Several studies have observed that most growth faltering is considered to happen during first 1,000 days and children who are stunted in this period may never meet their full physical and intellectual capability [14 59 60]. Early detection of change in attained growth for length-for-age could guide health care to implement adequate preventive and/or therapeutic measures to halt the growth retardation and prevent stunting [34].

WHO highlight that the most immediate causes of stunting are poor nutrition and repeated infections during early childhood [47]. The level of childcare and nutritional supplies in early life might vary according to the different levels of SES that in turn could have an impact on child linear growth or stunting [40].

A recent review of 256 article observed that among all measures of SES, mostly studies have used single component measures of SES such as assets, income, education, and occupation [54]. Very few studies have investigated multidimensional component measures of SES. Psaki et al. constructed a WAMI index as multidimensional component measure of SES and investigated its relationship with height-for-age in 8 low- and middle-income countries. The WAMI index could be a standardized approach to generalize SES across diverse population and warrants further investigation in other context [55].

Most studies have investigated child linear growth and its determinants among children under 5 years of age. Therefore, in light of importance of first 1,000 days in child linear growth, we aimed to investigate the association of a different measures of SES, including the multidimensional WAMI as well as a range of single component measures with length-for-age in children under 2 years of age in Nepal.

3. Aim and objective

To study the association of SES with linear growth in children under 2 years of age in Bhaktapur, Nepal.

Specific objective:

To assess the association of different measures of SES with attained linear growth assessed as length-for-age z-score calculated based on the WHO Child Growth Standards of children under 2 years of age in Bhaktapur, Nepal.

4. Materials and methods

4.1. Study Setting

Nepal is a small landlocked country in South Asia with an area of 147,181 km^2. It is bordered by India in the south, east, and west and by China in the north. Nepal is divided into three ecological zones: mountains, hills, and lowland plains. Elevations in Nepal range from 90 to 8,848 meters above sea level. Nepal is administratively divided into 7 provinces, where each province is further divided into districts and each district into municipalities [61]. In 2019, the United Nations Development Programme (UNDP) Human Development Index ranked Nepal at 147 out of 189 countries [62]. Some demographic statistics of the country are presented in Table 1.

Bhaktapur municipality (headquarter of Bhaktapur district) is our study area, which lies in province number 3 (Bagmati Pradesh) (Figure 2). This municipality is about 13 kilometres from the capital city of Nepal, Kathmandu. It lies in the hill zone at about 1,300 meter above sea level. The total population of Bhaktapur is more than 80,000, and is one of the most densely populated areas of Nepal. It is a peri-urban agricultural-based community with a population predominated by the Newar ethnic group. The local climate is humid subtropical with a wet and hot season (monsoon season) from May to August (with temperature reaching up to +35℃), and a dry and cool season from October to March (with temperature falling below -2℃). It is one of the main tourist attractions of Nepal with many traditional temples and historical buildings, which are on the UNESCO world heritage list. The main diet of people is bhat (rice), dal (pulse) and tarkari (vegetable).

Table 1. Nepal demographic statistics.

Indicators	**Statistics**
Total Population	30,266,743 [a]
Population density (per sq. kilometres)	196 [b]
Population growth (annual %)	1.8 [c]
Urban population (%)	20 [c]
Sex ratio (male per 100 female)	84.55 [d]
Life expectancy at birth (years)	70 [b]
Fertility rate (births per women)	1.92 [b]
Under-five mortality rate (per 1,000 live births)	30.8 [e]
Maternal mortality ratio (per 1,00,000 live birth)	186 [f]
Gross national income per capita (PPP US $)	3,610 [c]
Total expenditure on health per capita (US $)	57.85 [b]
Total expenditure on health (% of GDP)	5.84 [b]
Adult literacy rate (aged ≥15 Years, %)	67.9 [g]

Abbreviation- GDP (Gross development product), US (United States), PPP (Purchasing power parity)
[a] Central Bureau of Statistics (CBS), 2021 [63]; [b] World Bank, 2018 [64]; [c] World Bank, 2019 [64]; [d] United Nations (UN), 2020 [65]; [e] United Nations Children's Fund (UNICEF), 2019 [66]; [f] UNICEF, 2017 [66]; [g] United Nations Educational, Scientific and Cultural Organization (UNESCO), 2018 [67]

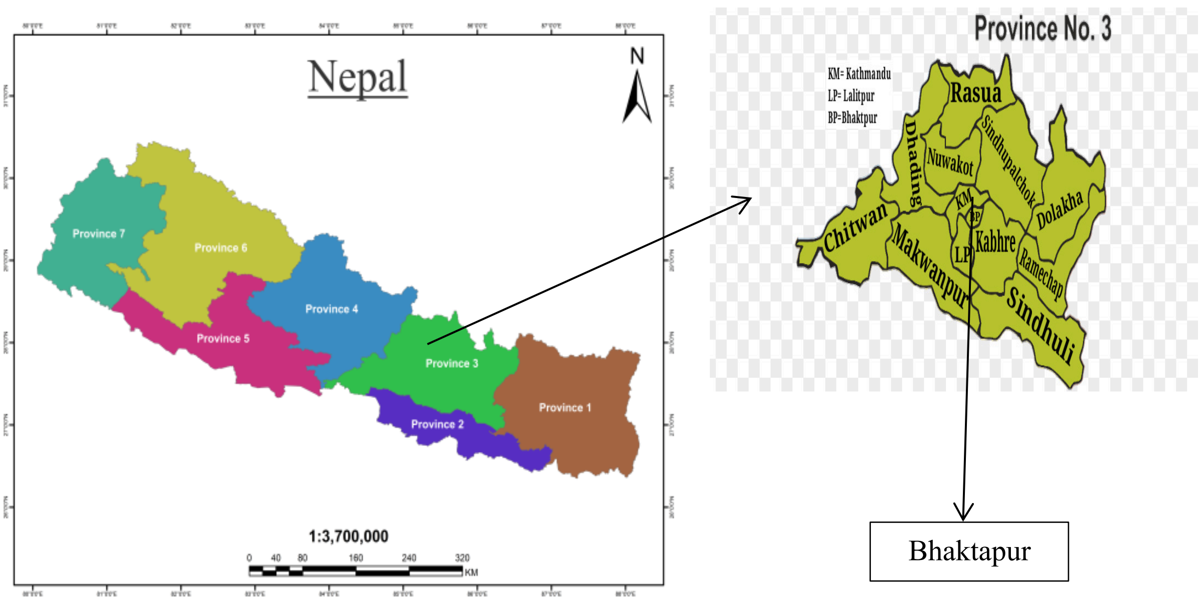

Figure 2. Map of Nepal showing all provinces with focus on province number 3 where our study site Bhaktapur is located [68].

4.2. Original study

4.2.1. Background

The main aim of the original study was to measure if daily supplementation with 2 micrograms (µg) of vitamin B12 for 12 months improved neurodevelopment, growth, and haemoglobin concentration in children aged 6-11 months at enrolment.

The original study was a superiority, parallel group, community-based, individually randomized, double-blind, placebo-controlled trial conducted in Bhaktapur, Nepal. All the children received an oral supplement of vitamin B12 or placebo every day for 12 months. The study enrolled the first child in April 2015 and enrolment ended in February 2017. Enrolment halted for one month because of an earthquake in Nepal. The enrolled children were followed-up for one year till March 2018. Details of the original study are published elsewhere [69].

4.2.2. Study population

The study population consist of 600 children aged 6-11 months who has length-for-age z-score < -1.

Exclusion criteria:

The following exclusion criteria were applied for the original study:

i) Severe systemic illness requiring hospitalization.

ii) Severe malnutrition (weight-for-length z-score < -3, referenced to the WHO Child Growth Standards of children for this age group). For ethical reasons, these children required micronutrient supplementation and adequate medical care.

iii) Lack of consent.

iv) Taking B vitamin supplements that include vitamin B_{12}

v) Severe anaemia (Hb<7 g/dl). This was a temporary exclusion criterion, and the children were enrolled if they had been successfully treated.

vi) Ongoing acute infection such as fever or infection that required medical treatment. This was a temporary exclusion criterion, and the children were enrolled after recovery.

4.3. Current study

4.3.1. Study population and design

The current cohort study includes all the 600 children aged 6-11 months with length-for-age z-score < -1 at the baseline, who were followed for 1 year (Figure 3). At the follow-up, 572 children aged 18-24 months remained in the study (loss to follow-up <5%).

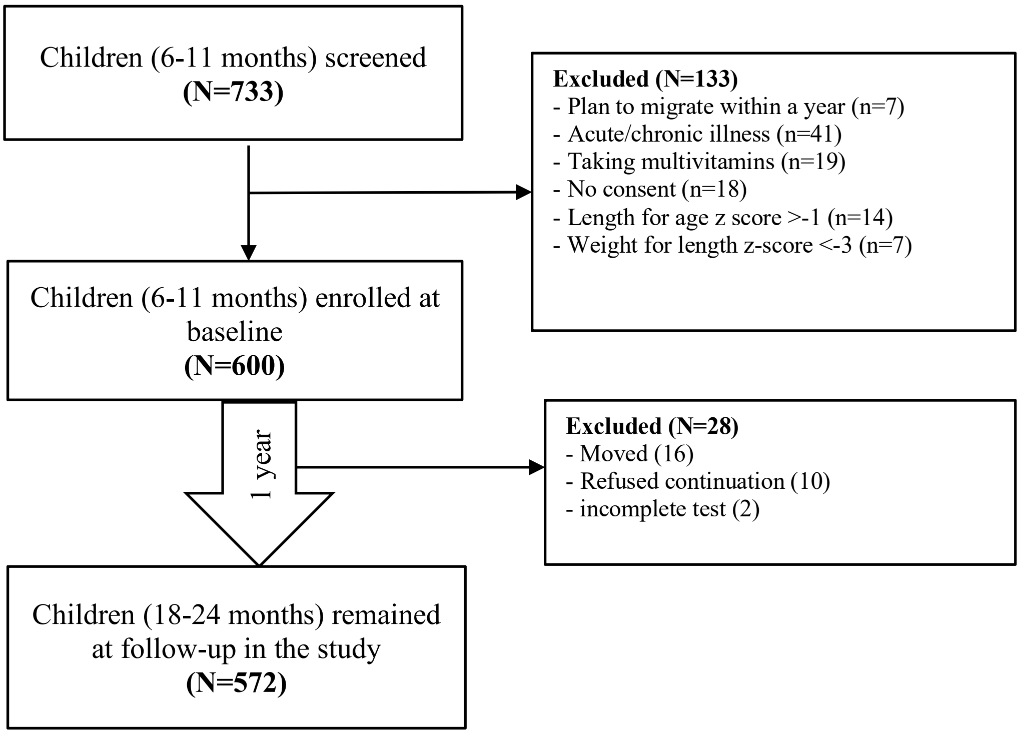

Figure 3. Flow chart of study participants.

4.3.2. Data collection and management

Child´s age was reported in completed months. Length and weight were measured in the clinic at baseline and at follow-up of the study. Length was measured in centimetres using a portable length board (model 417, Seca, California, USA). Weight was measured with a portable electronic scale that measured to the nearest 0.01 kg (model 877, Seca, California, United States of America). Child´s sex (male and female), birthweight (in grams), and gestational week were retrieved from hospital records. Mother's height (in centimetres) and weight (in kilograms) was measured at the clinic using a portable electronic scale (Salter/HoMedics Group, UK and seca, Germany) and a stadiometer (Prestige, Hardik Medi Tech, India). Furthermore, information about hospitalization of the child (yes and no), mother’s age (in years), type of family (nuclear and joint), ethnicity (Brahmin/Chettri, Newar, Tamang and other ethnic groups), father tobacco smoking, indoor tobacco smoking, separate bedroom and kitchen, type of cooking fuel, and other components of SES were assessed through interview with mother and father. Components of SES are described below in section 4.3.3.

All the collected data were checked manually by the supervisor for completeness and consistency. The data were double entered at the field clinic into appropriate databases with computerized range and consistency checks. The data cleaning process was continuous during the data collection and management period.

For this analysis, the principal investigator of the original project prepared a dataset in the research-based server SAFE at University of Bergen (UiB) and gave access to the data.

4.3.3. Study variables

For this study, we constructed a directed acyclic graph (DAG) to conceptualize a theoretical framework for the association between the exposure and the outcome including available variables in this study. The DAG was prepared using DAGitty software (Figure 4) [70].

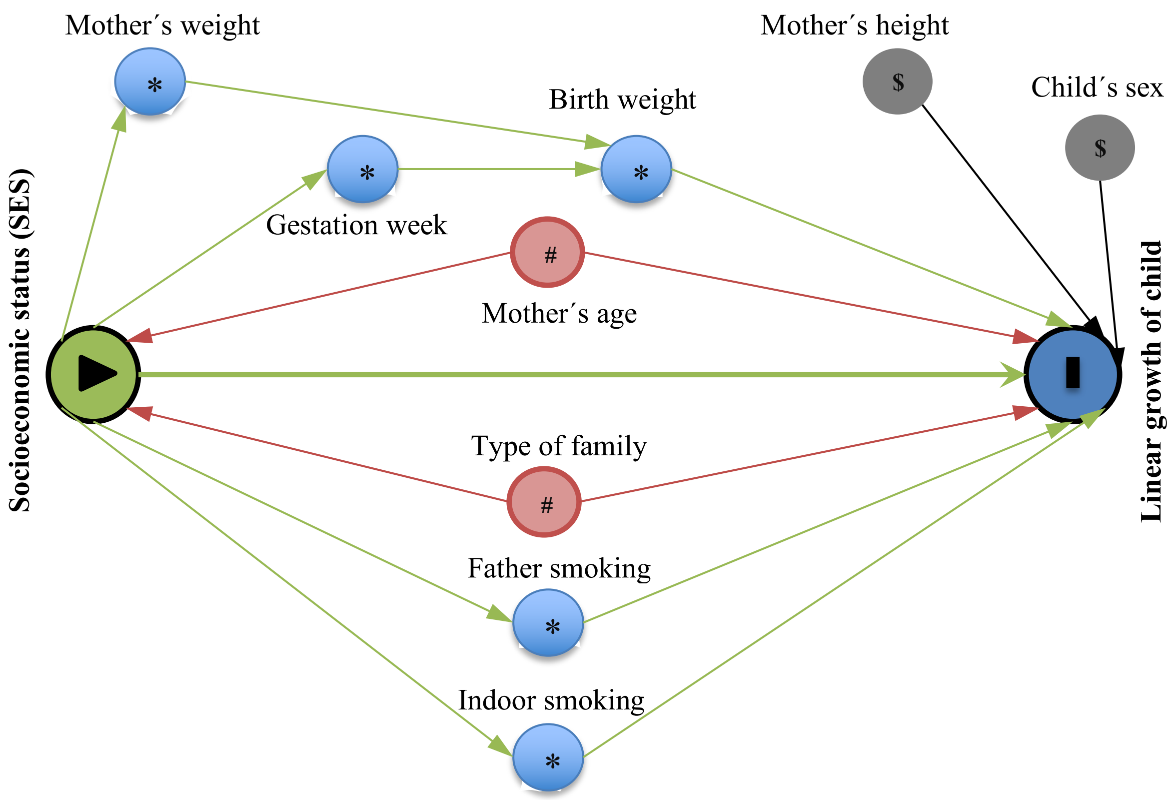

Figure 4. Directed acyclic graph (DAG) showing the role of all available study variables in this study.

Notes: ▶- exposure variable, ▮- outcome variable, #-confounder, *-mediator, $-competing exposure

i) Independent/exposure variables

SES was the main exposure variable, and we included a multidimensional measures and several single component measures of SES.

Multidimensional measure: WAMI index is a multidimensional measure of SES, which includes access to improved water and sanitation, assets, maternal education, and income. To assess safe drinking water, the WHO definition was adapted [71]. In addition to tap water, mineral water and water from water tanks were considered as safe drinking water in our context. The safe drinking water was categorized as yes and no. Similarly, for sanitation, toilet facilities with drainage and septic tank were considered safe [71]. The safe sanitation was categorized as yes and no. The assessment of assets included ownership of land, ownership of house, separate bedroom/kitchen, smoke free cooking fuel (gas and electricity), ownership of vehicle, tenant in house, and remittance from abroad. Each asset was categorized as yes and no. The overall asset was assessed by adding each asset on additive scale giving a range of 0-7. Maternal education was categorized as illiterate and primary education (≤5 years), secondary education and school leaving certificate (SLC)/Intermediate (6-12 years), bachelor and above (>12 years). Each individual measure was weighted equally to construct the WAMI index. The value for WAMI index ranges from 0 to 1, where a higher value indicates a higher SES. For our study purpose, we categorized the WAMI index into tertiles as low (0-0.57), medium (0.58-0.71), and high (0.72-1). Information on income was not available in our study, therefore the WAMI index was constructed without income.

Single component measure: The single component measures of SES were assets, mother´s and father´s education, mother´s and father´s occupation, safe drinking water, and safe sanitation. The asset ranges from 0 to 7 and was assessed as described above in the WAMI index. The asset was further categorized into tertiles as low (0-2 assets), medium (3-4 assets), and high (5-7 assets). Mother´s and father´s education was categorized as ≤5 years, 6-12 years, and ≥12 years as described above in the WAMI index. Mother´s and father´s occupation was categorized as daily wage earner, work in agriculture, and self-employed/services. The safe drinking water and safe sanitation were categorized as yes and no as described above in the WAMI index.

ii) Outcome variables

We used attained linear growth as outcome variable. The length was age- and sex-standardized by calculating the z-score of length-for-age using the WHO child growth standards [21]. The length-for-age was assessed at baseline (children aged 6-11 months) and at follow-up (children aged 18-24 months) in the study.

iii) Confounders

In the DAG framework, the confounders are the variables that are associated with both the exposure of interest and outcomes of interest, but not in the causal pathway. Using the DAG, the confounders identified were maternal age and type of family (Figure 4).

iv) Competing exposures

In the DAG framework, the competing exposure are the exposures that are associated with the outcomes of interest and have no path to and from the exposure of interest. In the DAG, the competing exposures explored for the association of SES with child linear growth were mother´s height and child sex (Figure 4).

4.3.4. Data analysis

Analysis was performed among 600 children at baseline (aged 6-11 months) and 572 at follow-up (aged 18-24 months). Data were analysed using R 4.0.2 software (http://www.r-project.org).

For the descriptive analysis of sample characteristics, mean and standard deviation (SD) (for normal distribution) or median and interquartile range (IQR) (for non-normal distribution) were calculated for continuous variables. Numbers (N) and percentages (%) were calculated for categorical variables.

To assess the association of the different measures for SES with length-for-age at baseline and at follow-up, separate linear regression models were built. The study had different measures of SES with different units; therefore, standardized regression coefficients with the corresponding 95% confidence intervals were calculated to get estimates comparable across all the measures of SES. For each measure of SES, three regression models were built, where Model 1 (univariable model) included only SES; Model 2 (multivariable model) was adjusted for the confounders mother´s age and types of family; Model 3 (multivariable model) was additionally adjusted for competing exposures mother's height and child´s sex. The adjustment of competing exposure in regression models might not introduce bias but could improve precision [70]. The selection of the variables included in the linear regression models was based on the DAG described in section 4.3.3 (Figure 4).

Additionally, a Model 4 (multivariable model) was built to assess the association of father´s education with child length-for-age adjusting for WAMI index in addition to adjusted variables in Model 3.

4.4. Ethics

Ethical approval for the original study was obtained from the Regional Committee for Medical and Health Research Ethics (REK number 2014/1528) in Western Norway and from the Nepal Health Research Council (NHRC number 233/2014) in Nepal. Written informed consent of children´s parent was obtained.

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The association between socioeconomic status and linear growth in Nepalese children under 2 years of age

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Abstract

Background WHO and UNICEF advocate the importance of the first 1,000 days (from conception to the 2^{nd} birthday) for a child´s optimum health, growth, and neurodevelopment. Mostly, studies have used single component measures of socioeconomic status (SES) such as assets, income, education, and occupation but few studies used multidimensional measures of SES. Therefore, we aimed to investigate the association of a range of measures of SES including multidimensional measures on linear growth among young Nepalese children.

Methods This study included 600 children under 2 years of age from Bhaktapur, Nepal. Linear growth was assessed as length-for-age z-score using the WHO child growth standard. Length was measured at baseline (child aged 6-11 months) and follow-up (child aged 18-24 months). Measures of SES assessed were a WAMI (water and sanitation, assets, maternal education) index, combined or as individual components, father´s education, and mother´s or father´s occupation. Linear regression models were employed to regress linear growth on the selected measures of SES.

Results There were 49% female children. At baseline, the mean age (SD) was 8 (1.8) months, the mean length-for-age z-score (SD) was -1.8 (0.6), and the mean (SD) WAMI index was 0.65 (0.14). At baseline, only mother's education was associated with length-for-age, whereas at follow-up, the WAMI index, parental education and occupation, and assets were associated with length-for-age when adjusted for mother's age and type of family. The estimates did not change considerably when adjusted for mother's height and child´s sex in addition.

Conclusion Different measures of SES were positively associated with linear growth among children under 2 years of age, where WAMI index, a multidimensional measure of SES, could be used as standardized approach to generalize SES of family across diverse population.

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Key questions

What is already known?

- Previous studies have investigated the role of socioeconomic status (SES) on linear growth particularly among children under 5 years of age.
- Most studies have used single component measures of SES such as assets, income, education, and occupation rather than multidimensional measures of SES such as WAMI index
- WAMI index was introduced in 2014 by the MAL-ED study spanning across 8 low- and middle-income countries.

What are the new findings?

- Among children aged 18-24 months, the WAMI index, parental education and occupation, and assets were associated with length-for-age when adjusted for mother's age and type of family.

What do the new findings imply?

- WAMI index, a multidimensional measure of SES, could be used as standardized approach to generalize SES of family across diverse population.

INTRODUCTION

Globally, 144 million children under 5 years of age were stunted in 2019, with the highest number of 56.9 million in South Asia [1]. In Nepal, among all forms of malnutrition in children under 5 years of age, stunting accounted for the largest proportion in 2016, with 36% being stunted and 12% severely so [2]. Stunting in early life has been associated with poor motor and cognitive development, as well as behavioural abnormality [3 4]. Long term consequences include shorter adult height, poor school achievement, low work productivity and low wages in adulthood, and increased risk of obesity and cardiovascular disease [5-7].

The World Health Organization (WHO) and United Nations Children's Fund (UNICEF) advocate that the first 1,000 days of life that starts from conception to the child 2nd birthday is a crucial time period for optimal physical and mental development, as well as for interventions to prevent stunting [8-11]. Several studies have observed that most growth faltering happens during this period and that stunting after 1,000 days is largely irreversible [12-14]. Children who become stunted during their first 1,000 days may never meet their full physical and intellectual capability [14]. WHO highlights that the most immediate causes of stunting are poor nutrition and repeated infections during early childhood [15]. The level of childcare and nutrition might vary according to the different levels of SES [16].

Socioeconomic status (SES) is commonly conceptualized as a combination of economic, social, and work status measured by income, wealth, education, or occupation, respectively [17 18]. A recent review of 256 articles observed that among all measures of SES, most studies used single component measures of SES such as assets, income, education, and occupation [19]. In 2014, the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study was conducted in 8 low- and middle-income countries. They introduced the WAMI (water and sanitation, assets, maternal education, and income) index as a multidimensional measure of SES which was suggested as a standardized approach to generalize SES across diverse population, warranting further investigation in other contexts [20].

We aimed to investigate the association of different measures of SES, including the WAMI index and single component measures, with linear growth assessed among children under 2 years of age in Nepal.

METHODS

Study population

This study used data from a completed randomized controlled trial conducted in Bhaktapur, Nepal, measuring the effect of vitamin B12 supplementation for 12 months on neurodevelopment, growth, and haemoglobin concentration in children [21]. All the children enrolled in the trial were included in this study. At baseline, 600 children aged 6-11 months with a length-for-age z-score < -1 were enrolled and followed for 1 year. At the end of the study, 572 children aged 18-24 months remained in the study; <5% were lost to follow-up (Figure 1).

Data collection and management

All the collected data were checked manually by supervisors for completeness and consistency. The data cleaning process was continuous during the data collection and management period.

Length and weight were measured in the study clinic at baseline and at end of the study. Length was measured in centimetres using a portable length board (model 417, Seca, California, USA). Weight was measured with a portable electronic scale that measured to the nearest 0.01 kg (model 877, Seca, California, United States of America). Length and weight were measured twice, and the mean values were used. Child´s age was reported in completed months. The length and weight were age- and sex-standardized by calculating the z-score for length-for-age, weight-for-age, and weight-for-length using the WHO child growth standard [22]. Child´s sex (male and female), birthweight (in grams), and gestational week were retrieved from hospital records. Mother's height (in centimetres) and weight (in kilograms) was measured at the clinic using a portable electronic scale (Salter/HoMedics Group, UK and seca, Germany) and a stadiometer (Prestige, Hardik Medi Tech, India). Furthermore, information about hospitalization of the child (yes and no), mother’s age (in years), type of family (nuclear and joint), ethnicity (Brahmin/Chettri, Newar, Tamang and other ethnic groups), father tobacco smoking, indoor tobacco smoking, separate bedroom and kitchen, type of cooking fuel, and other components of SES were assessed through interview with mother and father.

A range of measures of SES were assessed in this study, which includes a multidimensional and several single component measures of SES, the WAMI index. The original WAMI index includes access to improved water and sanitation, assets, mother´s education, and income [20]. We did not have information on income and thus considered the remaining 3 parts in the construction of the index. In addition to tap water as suggested by WHO, mineral water and water from water tanks were considered as safe drinking water in our study (categorized as yes and no) [23]. Similarly, for sanitation, toilet facilities with drainage and septic tank were considered safe (categorized as yes and no) [23]. The assessment of assets included ownership of land, ownership of house, separate bedroom/kitchen, smoke free cooking fuel (gas and electricity), ownership of vehicle, tenant in house, and remittance from abroad. Each asset was categorized as yes and no. Mother´s education was categorized as ≤5 years, 6-12 years, >12 years. Each of the 3 parts was weighted equally to construct the WAMI index. The value for WAMI index ranges from 0 to 1, where a higher value indicates a higher SES. For our study purpose, we categorized the WAMI index into tertiles, i.e., low (0-0.57), medium (0.58-0.71), and high (0.72-1).

The single component measures were assets, mother´s and father´s education, mother´s and father´s occupation, drinking water supply, and toilet facility. Mother´s and father´s occupation was categorized as daily wage earner, work in agriculture, and self-employed/services. The sum of assets was further categorized into tertiles: low (0-2 assets), medium (3-4 assets), and high (5-7 assets).

Data Analysis

Data were analysed using R 4.0.2 software (http://www.r-project.org).

For the descriptive analysis of sample characteristics, mean and standard deviation (SD) (for normal distribution) or median and interquartile range (IQR) (for non-normal distribution) were calculated for continuous variables. Numbers (N) and percentages (%) were calculated for categorical variables.

To assess the association of the different measures for SES with length-for-age at baseline and at follow-up, separate linear regression models were built. The study had different measures of SES with different units; therefore, standardized regression coefficients with the corresponding 95% confidence intervals were calculated to get estimates comparable across all the measures of SES. Separately for each measure of SES, three regression models were built, where Model 1 (univariable model) included only SES; Model 2 (multivariable model) was adjusted for the confounders mother´s age and type of family; Model 3 (multivariable model) was additionally adjusted for competing exposures mother's height and child´s sex. The adjustment of competing exposure in regression models could improve precision of the estimate of association [24]. The selection of the variables included in the linear regression models was guided by data availability and conceptualization using a Directed Acyclic Graph (DAG).

Additionally, a Model 4 (multivariable model) was built to assess the association of father´s education with child height-for-age adjusting for WAMI index in addition to adjusted variables in Model 3.

Ethics

Ethical approval for the original study was obtained from the Regional Committee for Medical and Health Research Ethics (REK number 2014/1528) in Western Norway and from the Nepal Health Research Council (NHRC number 233/2014) in Nepal. Written informed consent of children´s parent was obtained.

RESULTS

This analysis includes 600 children under 2 years of age. There were 49% female children. The mean age (SD) at baseline was 8 (1.8) months and at follow-up it was 20 (1.8) months. The mean length-for-age z-score of children was -1.8 both at baseline and follow-up. The mean age (SD) of mothers was 27 (4.7) years, and most belonged to the Newar ethnic group (70%) (Table 1).

The mean (SD) WAMI index, was 0.65 (0.14). Among the 7 types of family assets, the median (IQR) number of assets was 3 (2-4) (Table 1). About one-third of the mothers and fathers were illiterate or had ≤5 years of education (37% and 36%, respectively). The majority of the mothers (62%) were working within the field of agriculture; around half of the fathers (40%) were daily wage earners. Around 19% of the families were using biomass cooking fuel and about half of the families (50%) had common bedroom and kitchen (Table 1).

At baseline, only mother's education was associated with length-for-age z-score, when adjusted for mother's age and type of family (Table 2, Model 2). Children whose mother had an education >12 years had on average a 0.34 higher length-for-age z-score compared to children whose mother had an education ≤5 years (Model 2). The estimate did not change considerably when additionally adjusted for mother's height and child´s sex (Table 2, Model 3).

At follow-up, the WAMI index, parental education and occupation, and assets were associated with length-for-age z-score when adjusted for mother's age and type of family (Model 2). Comparing the highest (3rd) to the lowest (1st) tertile, the average length-for-age z-score of children was 0.34 higher for WAMI index, and 0.25 higher for assets (Model 2). Comparing >12 years of education to ≤5 years, the average length-for-age z-score of children was 0.39 higher for mothers and 0.51 higher for fathers (Model 2). Furthermore, comparing self-employed mothers to daily wage earners, the average length-for-age z-score of children were 0.33 higher; for fathers it was 0.28 higher (Model 2) (Table 2). The estimate did not change considerably when adjusted for mother's height and child´s sex in addition (Model 3). There was no association of drinking water supply or toilet facility with length-for-age z-score of the children (Table 2).

As father's education was not included in the WAMI index, we built an additional regression model adjusting for WAMI index in addition to variables in Model 3. The estimates remained almost unchanged at both baseline (>12 years vs. ≤5 years, b=0.22, 95% CI -0.05 to 0.50) and follow-up (>12 years vs. ≤5 years, b=0.39, 95% CI 0.11 to 0.67).

DISCUSSION

In this study, several indicators of SES, assessed when the child was 6-11 months old, were associated with linear growth 1 year later, namely, the WAMI index, number of assets, as well as parental education and occupation group.

Psaki et al. found that a 25% difference in the WAMI index score was associated with an increase of 0.38 SD in height-for-age among children 24-60 months of age [20]. Another study within the MAL-ED network showed that a low WAMI index (1th quartile) was associated with 1.75 times higher risk of stunting in children at 24 months compared to high WAMI index (4st quartile) [25]. Similar to these studies, although we had no information on income, we found that families with a high WAMI index (3rd tertile) had higher length-for-age compared to families with a low WAMI index (1st tertile). Only a few other published studies used the WAMI index, and found an association with different outcomes, such as cognitive development in children and dietary diversity in adults [26 27]. In developing countries, a single component measure of SES might not capture well the household SES reflecting the complex social structure. Our study supports the use of the WAMI index in the low- and middle-income setting. The WAMI index could be used as a standardized approach to generalize SES of family across diverse population [20].

Although a single component measures of SES might not reflect the overall household SES, investigation of these measures could help to pinpoint the main drivers and could guide in designing and implementing health care interventions. Several studies have used single component measures such as assets, mother´s education, father´s education, mother´s occupation, father´s occupation, safe drinking water supply, and toilet facility, to investigate the socioeconomic inequalities in heath and its related outcomes. Similar to our study, a systematic review reported that a higher number of assets was associated with height-for-age in children under 5 years of age in developing countries including Cambodia, Pakistan, and Bangladesh [28]. In low- and middle-income countries such as Nepal, data on income and expenditure are not easily available and many people have various sources of income including cash crops. Therefore, manifest variables such as the number of assets could be helpful to obtain SES of family [29-31].

Our study observed that parent's education and employment was positively associated with length-for-age among children under 2 years of age. Similar to our study finding, in a study conducted in Bangladesh, India, Nepal, and Pakistan, higher parental education was associated with higher height-for-age among children under 5 years of age [32]. In a study conducted in Indonesia and Bangladesh, the increase in years of parental education was associated with a decreased risk of stunting in children under 5 years of age [33]. Similar associations with parental education have been reported in several other studies in low- and middle-income countries among children under 5 years of age [28 32 34 35]. Regarding the parental occupation, a study performed in Uganda observed that mothers who had professional job had children with a lower risk of stunting compared to mothers who had a manual job [36]. Additionally, in a study by WHO in sub-Saharan Africa children of mothers who were in professional, technical and managerial work had a lower risk of stunting compared to children of mothers who were in sales, service, agriculture, or household and domestic work [37]. Very few studies have assessed the association with father´s occupation. In contrast to our study, two studies from Indonesia did not find any association. However, Ramli et al. only presented results from univariable analysis and Gunardi et al. had small sample size (N=160) resulting in largely imprecise results [38 39]. Parental education and employment are considered as important components of SES determining the health and wellbeing of a family. Educated parents are potentially in a better position to provide care and nutrition to their children, underpinned by better and more autonomous health care decisions [40]. Mothers who have better employment and education are likely to have a better decision-making power for household purchase, freedom of mobility, and a healthy diet in the family [41 42].

A study in East Africa showed that safe drinking water and sanitation was associated with higher length-for-age among children 6-24 months of age [43]. Similarly, another study in Sudan found that risk of stunting was higher in children (aged 6-72 months) with families who had poor drinking water and sanitation facility [44]. In contrast to these studies, we did not find an association of drinking water supply and sanitation facility with length-for-age among children under 2 years of age. In our study, only a few families had unsafe drinking water and no toilet facility, and thus, there was little variation in the exposure itself. In the recent years, tremendous improvements in sanitation and hygiene have been made in Nepal [45] which might indicate that safe water and sanitation is no longer a main driver of impaired linear length.

WHO advocates the importance of the first 1,000 days for child growth, which is a crucial time period from conception to the 2nd birthday [11]. During these first 1,000 days, good nutrition and health care build a strong foundation for optimal health, growth, and neurodevelopment. It is also suggested as a crucial time for interventions in order to prevent stunting because most growth faltering is considered to happen during this period and stunting after 1,000 days is almost irreversible [12 13 46]. Studies have reported a high prevalence of stunting in children within the age group 6-20 months and in children whose family has only few assets [47-49]. Therefore, the investigation of growth determinants such as SES during this period is important. Global targets including sustainable development goals and most of the studies performed so far have focused on children under 5 years. However, our study investigated the SES in relation to child linear growth during the important first 1,000 days period.

Our study has several strengths. We believe that the sample size (N=600) is adequate to observe clinical relevant observations and the proportion of participants lost during the 1-year follow-up was minimal (<5%). The anthropometric measurements were taken by thoroughly trained study personnel and were thus considered less prone to measurement errors. We report standardized regression coefficients to make the associations of the different indicators comparable. The selection of confounders was based on a conceptual framework using DAG; however, unmeasured confounding cannot be excluded.

The study has certain limitations. This study enrolled children who were marginally stunted (length-for-age z-score < -1). This limits the generalizability of our results. The restricted variation in the outcome and exposure variables could have led to underestimation of some of the associations.

Conclusions

In this study, SES was positively associated with linear growth among children under 2 years of age. WAMI index, a multidimensional measure of SES, could be used as standardized approach to generalize SES of family across diverse population in low- and middle-income countries.

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Competing interest

None declared

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Figure 1: Flow chart of study participants.

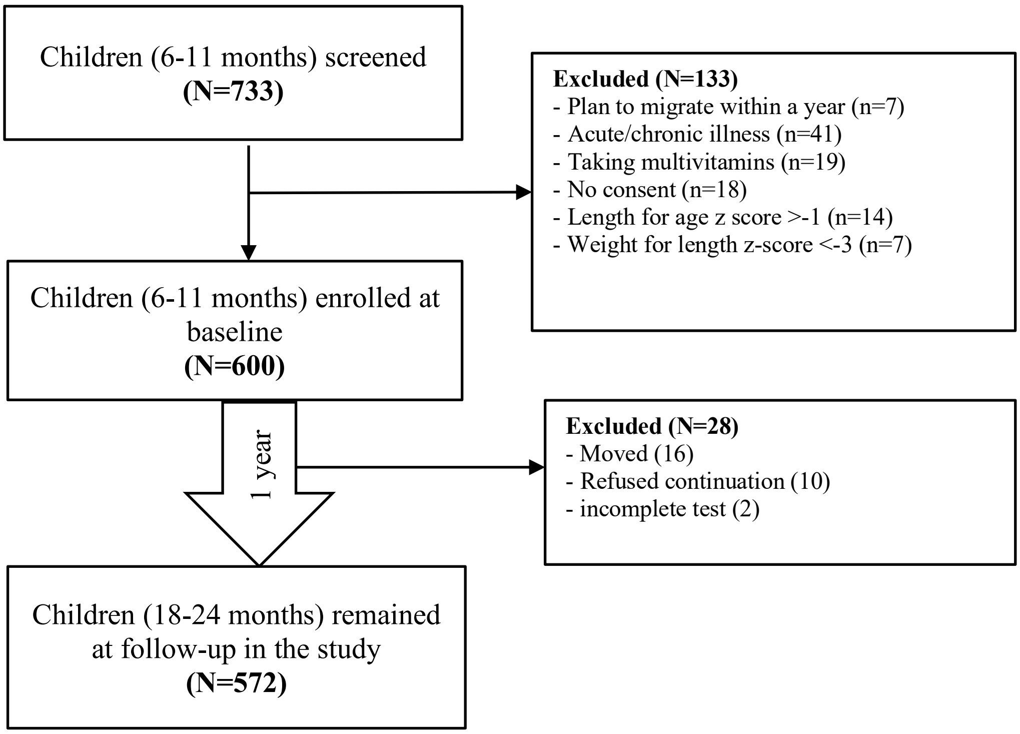
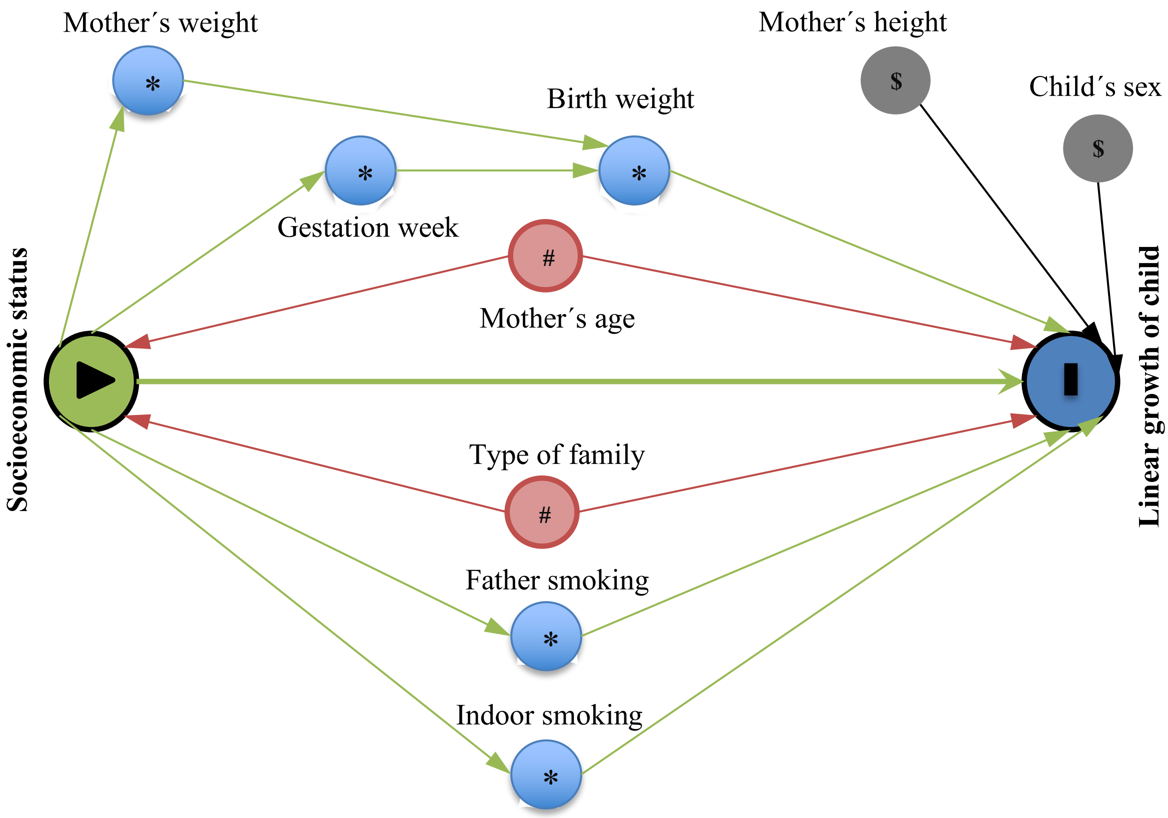


Figure 2: Directed Acyclic Graph (DAG) showing the role of all available study variables in this study.



Notes: ▶- exposure variable, ▮- outcome variable, #-confounder, *-mediator, $-competing exposure

Table 1. Descriptive characteristics of children under 2 years of age in Bhaktapur, Nepal.

Characteristics (N=600)	**N (%)**	**Mean (SD)**
Child		
Sex (female)	291 (48.5)	
Age (baseline, in months)		8.0 (1.8)
Age (follow-up, in months)		20.0 (1.8)
Birthweight (in gm)		3040 (1413.4)
Length-for-age z-score (baseline)		-1.8 (0.59)
Length-for-age z-score (follow-up) *		-1.8 (0.67)
Weight-for-age z-score (baseline)		-1.3 (0.85)
Weight-for-age z-score (follow-up) *		-1.4 (0.75)
Weight-for-length z-score (baseline)		-0.2 (0.96)
Weight-for-length z-score (follow-up) *		-0.7 (0.83)
Hospitalization in first month	54 (9.0)	
Hospitalization after first month	26 (4.3)	
Pre-term birth (before 37 weeks of gestation) *	62 (10.2)	
Family		
Age of mother (in years)		27.3 (4.7)
Weight of mother (in kg)		53.5 (8.6)
Height of mother (in cm)		150.1 (5.3)
Type of family (Joint)	292 (48.7)	
Ethnicity		
Brahmin/Chhetri	47 (7.8)	
Newar	422 (70.3)	
Tamang and other ethnic groups	131 (21.8)	
Father tobacco smoking (current) *	207 (42.4)	
Indoor tobacco smoking	303 (50.5)	
Socioeconomic status		
WAMI index		0.65 (0.14)
Assets		3 (2) #
Mother´s education		
≤5 years	223 (37.2)	
6-12 years	261 (43.5)	

>12 years	116 (19.3)	
Father´s education *		
≤5 years	212 (35.3)	
6-12 years	280 (46.7)	
>12 years	107 (17.8)	
Mother´s occupation *		
Daily wage earner	90 (15.0)	
Agriculture	373 (62.2)	
Self-employed/services	136 (22.7)	
Father´s occupation *		
Daily wage earner	240 (40.0)	
Agriculture	34 (5.7)	
Self-employed/services	324 (54.0)	
Own land	282 (47.0)	
Own house	309 (51.5)	
Own vehicle	252 (42.0)	
Remittance	57 (9.5)	
Tenants	65 (10.8)	
Bedroom and kitchen (not separated)	298 (49.7)	
Type of cooking fuel (smoke)	114 (19.0)	
Drinking water supply (no)	21 (3.5)	
Place of defecation (no toilet facility)	18 (3.0)	

*missing [gestation weeks=1, father tobacco smoking=4, mother´s occupation=1, father´s occupation=2, father´s education=1, Length-for-age z-score (follow-up)=28, Weight-for-age z-score (follow-up)=28, Weight-for-length z-score (follow-up)=28]

#Median (Inter quartile range)

Table 2. Association of socioeconomic status with child length-for-age at 6-11 months (baseline) and 18-24 months (follow-up) of age in Bhaktapur, Nepal.

Socioeconomic status	**N**	**Length-for-age** (N=600) (baseline, 6-11 months)			**Length-for-age** (N=572) (follow-up, 18-24 months)		
		Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
		b (95% CI)	*b* (95% CI)	*b* (95% CI)	*b* (95% CI)	*b* (95% CI)	*b* (95% CI)
WAMI index							
Low (1st tertile)	203	Ref	Ref	Ref	Ref	Ref	Ref
Medium (2nd tertile)	221	0.05(-0.14,0.24)	0.08(-0.12,0.27)	0.01(-0.18,0.19)	0.14(-0.05,0.34)	0.17(-0.03,0.37)	0.12(-0.08,0.31)
High (3rd tertile)	176	-0.02(-0.22,0.19)	0.06(-0.17,0.28)	-0.02(-0.24,0.20)	0.25(0.05,0.46)	0.34(0.11,0.56)	0.26(0.04,0.48)
Assets							
Low (1st tertile)	266	Ref	Ref	Ref	Ref	Ref	Ref
Medium (2nd tertile)	212	-0.07(-0.25,0.11)	-0.05(-0.24,0.15)	-0.06(-0.25,0.13)	0.18(-0.00,0.37)	0.24(0.04,0.44)	0.22(0.03,0.42)
High (3rd tertile)	122	-0.12(-0.34,0.09)	-0.08(-0.32,0.16)	-0.09(-0.32,0.14)	0.17(-0.05,0.39)	0.25(0.01,0.50)	0.23(-0.01,0.47)
Mother´s education							
≤5 years	223	Ref	Ref	Ref	Ref	Ref	Ref
6-12 years	261	0.17(-0.01,0.35)	0.21(0.03,0.39)	0.15(-0.03,0.32)	0.23(0.05,0.41)	0.26(0.07,0.44)	0.20(0.02,0.39)
>12 years	116	0.26(0.04,0.49)	0.34(0.11,0.57)	0.28(0.05,0.51)	0.34(0.11,0.57)	0.39(0.15,0.62)	0.33(0.10,0.56)
Father´s education							
≤5 years	212	Ref	Ref	Ref	Ref	Ref	Ref
6-12 years	280	0.20(0.03,0.38)	0.24(0.06,0.42)	0.16(-0.02,0.34)	0.28(0.10,0.47)	0.31(0.13,0.49)	0.24(0.06,0.42)
>12 years	107	0.17(-0.07,0.40)	0.24(-0.01,0.48)	0.15(-0.08,0.39)	0.45(0.22,0.69)	0.51(0.27,0.75)	0.43(0.19,0.67)
Mother´s occupation							
Daily wage earner	90	Ref	Ref	Ref	Ref	Ref	Ref
Agriculture	373	-0.09(-0.32,0.14)	-0.05(-0.29,0.18)	-0.02(-0.24,0.21)	0.04(-0.20,0.27)	0.07(-0.17,0.31)	0.07(-0.16,0.31)
Self employs/services	136	0.12(-0.15,0.38)	0.19(-0.09,0.46)	0.20(-0.06,0.47)	0.28(0.01,0.55)	0.33(0.05,0.61)	0.30(0.03,0.58)
Father´s occupation							
Daily wage earner	240	Ref	Ref	Ref	Ref	Ref	Ref
Agriculture	34	-0.08(-0.44,0.28)	-0.05(-0.41,0.32)	-0.02(-0.38,0.32)	0.34(-0.03,0.71)	0.37(0.00,0.75)	0.39(0.02,0.75)
Self employs/services	324	0.11(-0.06,0.28)	0.14(-0.03,0.31)	0.12(-0.05,0.28)	0.26(0.09,0.43)	0.28(0.10,0.45)	0.25(0.08,0.42)
Drinking water supply							
No	21	Ref	Ref	Ref	Ref	Ref	Ref

Yes	579	-0.02(-0.45,0.42)	-0.03(-0.46,0.41)	-0.12(-0.55,0.30)	0.05(-0.39,0.49)	0.05(-0.39,0.48)	-0.04(-0.47,0.39)
Toilet facility							
No	18	Ref	Ref	Ref	Ref	Ref	Ref
Yes	582	-0.09(-0.56,0.38)	-0.08(-0.54,0.39)	-0.18(-0.64,0.27)	-0.11(-0.59,0.38)	-0.10(-0.59,0.38)	-0.26(-0.73,0.22)

Abbreviations: WAMI (water and sanitation, assets, maternal education, income), *b* (regression coefficient), CI (confidence interval), N (number)
Model 1- included socioeconomic status; Model 2- includes socioeconomic status, mother´s age, and type of family; Model 3- includes socioeconomic status, mother´s age, type of family, mother´s height, and child´s sex.
Assets- included land ownership, house ownership, vehicle ownership, separate bedroom and kitchen, smoke free cooking fuel, tenants, and remittance.
WAMI index - included water and sanitation, assets, and mother´s education.

Annexes

1. Original study
2. Ethical clearance for original study:
   a) Nepal Health Research Council (NHRC)
   b) Regional Committees for Medical and Health Research Ethics (REK)
3. Permission from Elsevier for Figure 1
4. Information for Authors for BMJ Global Health

PLOS MEDICINE

RESEARCH ARTICLE

Effects of vitamin B_{12} supplementation on neurodevelopment and growth in Nepalese Infants: A randomized controlled trial

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Abstract

Background

Vitamin B_{12} deficiency is common and affects cell division and differentiation, erythropoiesis, and the central nervous system. Several observational studies have demonstrated associations between biomarkers of vitamin B_{12} status with growth, neurodevelopment, and anemia. The objective of this study was to measure the effects of daily supplementation of vitamin B_{12} for 1 year on neurodevelopment, growth, and hemoglobin concentration in infants at risk of deficiency.

Methods and findings

This is a community-based, individually randomized, double-blind placebo-controlled trial conducted in low- to middle-income neighborhoods in Bhaktapur, Nepal. We enrolled 600 marginally stunted, 6- to 11-month-old infants between April 2015 and February 2017. Children were randomized in a 1:1 ratio to 2 μg of vitamin B_{12}, corresponding to approximately 2 to 3 recommended daily allowances (RDAs) or a placebo daily for 12 months. Both groups were also given 15 other vitamins and minerals at around 1 RDA. The primary outcomes were neurodevelopment measured by the Bayley Scales of Infant and Toddler Development 3rd ed. (Bayley-III), attained growth, and hemoglobin concentration. Secondary outcomes included the metabolic response measured by plasma total homocysteine (tHcy) and methylmalonic acid (MMA). A total of 16 children (2.7%) in the vitamin B_{12} group and 10 children (1.7%) in the placebo group were lost to follow-up. Of note, 94% of the scheduled daily doses of vitamin B_{12} or placebo were reported to have been consumed (in part or completely). In this study, we observed that there were no effects of the intervention on the Bayley-III scores, growth, or hemoglobin concentration. Children in both groups grew on an average 12.5 cm (SD: 1.8), and the mean difference was 0.20 cm (95% confidence interval

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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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: AMC and PMU are paid employees at Bevital AS.

Abbreviations: Bayley-III, Bayley Scales of Infant and Toddler Development 3rd ed.; CI, confidence interval; CONSORT, CONsolidated Standards of Reporting Trials; GC-MS/MS, gas chromatography-tandem mass spectrometry; GMR, geometric mean ratio; GSDF, geometric standard deviation factor; ICC, intra-class correlation coefficient; id, identification; IMCI, Integrated Management of Childhood Illness; MMA, methylmalonic acid; NHRC, Nepal Health Research Council; RDA, recommended daily allowance; RCT, randomized controlled trial; REC, Regional Committee for Medical and Health Research Ethics; tHcy, total Homocysteine; 3cB12, combined indicator of cobalamin status.

(CI): −0.23 to 0.63, $P = 0.354$). Furthermore, at the end of the study, the mean difference in hemoglobin concentration was 0.02 g/dL (95% CI: −1.33 to 1.37, $P = 0.978$), and the difference in the cognitive scaled scores was 0.16 (95% CI: −0.54 to 0.87, $P = 0.648$). The tHcy and MMA concentrations were 23% (95% CI: 17 to 30, $P < 0.001$) and 30% (95% CI: 15 to 46, $P < 0.001$) higher in the placebo group than in the vitamin B_{12} group, respectively. We observed 43 adverse events in 36 children, and these events were not associated with the intervention. In addition, 20 in the vitamin B_{12} group and 16 in the placebo group were hospitalized during the supplementation period. Important limitations of the study are that the strict inclusion criteria could limit the external validity and that the period of vitamin B_{12} supplementation might not have covered a critical window for infant growth or brain development.

Conclusions

In this study, we observed that vitamin B_{12} supplementation in young children at risk of vitamin B_{12} deficiency resulted in an improved metabolic response but did not affect neurodevelopment, growth, or hemoglobin concentration. Our results do not support widespread vitamin B_{12} supplementation in marginalized infants from low-income countries.

Trial registration

ClinicalTrials.gov NCT02272842

Universal Trial Number: U1111-1161-5187 (September 8, 2014)

Trial Protocol: Original trial protocol: PMID: 28431557 (reference [18]; study protocols and plan of analysis included as Supporting information).

Author summary

Why was this study done?

- Many marginalized children fail to reach their cognitive and growth potential.
- Subclinical vitamin B_{12} deficiency, which is poor vitamin B_{12} status without overt clinical symptoms, is common in this population in Nepal.
- Vitamin B_{12} deficiency in children is associated with anemia (low hemoglobin concentration), stunted growth, and poor neurodevelopment.

What did the researchers do and find?

- In this population-based, double-blind, randomized controlled trial (RCT), we measured the effects of daily supplementation of vitamin B_{12} for 1 year in 600 infants.
- The primary outcomes were neurodevelopment, growth, and hemoglobin concentration.
- We targeted stunted infants as these children are at risk of vitamin B_{12} deficiency.
- Daily supplementation of vitamin B_{12} for a year resulted in a metabolic profile reflecting substantially improved B_{12} status (lower total homocysteine (tHcy) and methylmalonic acid

(MMA) concentrations) but did not affect neurodevelopment, growth, or hemoglobin concentration.

What do these findings mean?

- In spite of the improved metabolic profile following vitamin B_{12} supplementation, the findings do not support widespread vitamin B_{12} supplementation to improve short-term growth, neurodevelopment, or hemoglobin concentration in infants.

Introduction

Vitamin B_{12} (cobalamin) deficiency is common and affects all ages worldwide [1–3]. Animal-based foods are the primary sources, and poor status is prevalent in South Asia as well as in other low- and middle-income regions with low consumption of meat, animal milk, and fish [3,4].

Vitamin B_{12} is required for cell division and differentiation, utilization of energy, and other critical metabolic processes [5–7]. Failure to thrive, delayed development, and macrocytic anemia are typical manifestations in children with severe deficiency [8]. Several observational studies have demonstrated associations between biomarkers of vitamin B_{12} status with growth, neurodevelopment, and anemia [1,9–14]. The results from these studies suggest that the negative consequences of poor B_{12} nutrition are also seen in suboptimal B_{12} status, and not only in those with clinical deficiency. These observations, however, may be due to factors such as unmeasured confounding or limitations in the biomarkers used to assess vitamin B_{12} status.

Three randomized controlled trials (RCTs) have measured the effect of vitamin B_{12} supplementation on neurodevelopment in children with suboptimal B_{12} status. Of these, 2 facility-based RCTs in infants born at low birth weight or who had developmental delay showed that a high dose of vitamin B_{12} (400-μg hydroxycobalamin intramuscularly) substantially improved motor development [15,16]. In the third study, a population-based RCT in young North Indian children, there was a small borderline significant beneficial effect of daily oral supplementation with 1.8 μg of vitamin B_{12} for 6 months on neurodevelopment [17]. The evidence from these RCTs support the notion that poor vitamin B_{12} status may be a relevant public health concern; however, the evidence is currently not strong enough to alter widespread feeding recommendations. We therefore designed the current population-based RCT to measure the effects of daily supplementation of vitamin B_{12} for 1 year. In this study, we ensured that the baseline B_{12} status and metabolic response to the supplementation was well characterized, and we targeted marginally stunted infants due to their associated risk of vitamin B_{12} deficiency, delayed development, and stunted growth.

Participants and methods

Study design and participants

This study is reported according to the CONsolidated Standards of Reporting Trials (CONSORT) guidelines (S1 Checklist). The study was a community-based, double-blind placebo-controlled trial in Nepalese infants. We hypothesized that daily administration of 2 μg vitamin B_{12} for 12 months would improve neurodevelopment, growth, and hemoglobin concentration. We conducted the study in Bhaktapur municipality and surrounding peri-urban communities near the capital Kathmandu. For the last 20 years, we have shown that poor vitamin B_{12} status is common in both women and children in this population [4,18,19]. Bhaktapur is among the most densely populated municipalities in Nepal. The majority of births (96%) occur at health centers, and 1 in every second family is living in joint households and has a separate kitchen [20]. The primary outcomes were neurodevelopment measured by the Bayley Scales of Infant and Toddler Development 3rd ed. (Bayley-III), attained growth (cm and kg), and hemoglobin concentration. The secondary outcome was the metabolic response measured by plasma concentrations of cobalamin, total homocysteine (tHcy), and methylmalonic acid (MMA).

Ethics statement

The study received ethical clearance from the Nepal Health Research Council (NHRC; #233/2014) and from the Regional Committee for Medical and Health Research Ethics (REC; #2014/1528) in Norway. After detailed information was provided to parents, we obtained written informed consent or a thumbprint from those who were illiterate (in the presence of an impartial witness).

Enrollment, randomization, and blinding

We enrolled 600 children between April 17, 2015 and February 15, 2017. Inclusion criteria were age 6 to 11 months, length for age z-score <-1, intent to reside in the municipality and surrounding areas for the next 12 months, and availability of informed consent from parents. Children were excluded if they were taking (or planned to take) supplements that contained vitamin B_{12}, had a severe systemic illness requiring hospitalization, if they were severely malnourished (weight for length z-score <-3), were severely anemic (hemoglobin concentration <7 g/dL), or had ongoing infections that required medical treatment. In cases of severe malnutrition, anemia, or infections, children received treatment and were screened again for eligibility after recovery.

Field workers identified eligible children from immunization clinics or through home visits. Infants were enrolled by a study supervisor or by a physician at the field office. We randomized the infants in a 1:1 ratio in blocks of 8 using a computer-generated randomization list. Randomization was concealed, and the study double-blinded as the participants were only linked to the intervention through the identification (id) number printed on the supplement labels. The list that linked this id number to the randomization code was kept with the producers of the supplements and the scientist who generated it. None of the investigators had access to this list until the data collection and cleaning for the primary outcomes were completed. The vitamin B_{12} supplements and placebo were produced specifically for the trial and were identical in taste and appearance. At enrollment and end of study, we measured neurodevelopment, weight, length, and hemoglobin concentration.

Intervention and co-interventions

All children received 2 μg of vitamin B_{12} (cyanocobalamin), corresponding to approximately 2 to 3 recommended daily allowances (RDAs) or placebo via a daily oral supplement for 12 months. The intervention was implemented using sachets containing 20 grams of a lipid-based paste produced by GC Rieber Compact (Gurgaon, Haryana, India; http://www.gcrieber-compact.com/). Each sachet provided the daily dose of supplements. To ensure that the effect of vitamin B_{12} was not limited by inadequate intake of other essential nutrients, both the placebo and vitamin B_{12} paste contained a base multi-micronutrient mixture with several other vitamins and minerals at approximately 1 RDA. All caregivers were given dietary recommendations according to national guidelines. Children who developed diarrhea during the intervention period received zinc and oral rehydration solution. Those with mild to moderate anemia (hemoglobin 7 to 10 g/dL) were treated with per oral iron for at least 30 days. Children with pneumonia, dysentery, or other illnesses were treated according to the most recent Integrated Management of Childhood Illness (IMCI) guidelines [21].

During weekly visits to the homes, field workers asked the mothers about intake of the paste during the past 7 days and recorded in detail the amount of paste given to the children (i.e., half, one-third, three-fourths, or less). All episodes of vomiting or regurgitation after supplementation of the paste were also recorded, and the total number of empty paste sachets were counted at the weekly visits to verify the reported compliance.

Outcomes

Neurodevelopment

The Bayley-III is a comprehensive assessment tool of neurodevelopment in infants and toddlers aged 1 to 42 months [22]. The Bayley-III is often regarded as the gold standard for assessing neurodevelopment in this age-group and is used in research worldwide. We administered the Bayley-III directly with the child at enrollment and end of the study at the study research office. The Bayley-III consists of a cognitive, language (receptive and expressive), motor (fine and gross motor), and socio-emotional scale. Three psychologists, of whom 1 had extensive experience with the Bayley-III, were trained to perform the assessments for the study. To ensure high-quality measurements, we performed standardization exercises in 20 children ahead of the enrollment where the Bayley assessments were scored by 2 raters. The psychologists were required to reach an intra-class correlation coefficient (ICC) >0.90 with the expert rater, who served as the gold standard. Of note, 7% of the sessions during the main study were double scored by the expert rater to ensure appropriate interobserver agreement throughout the study. The ICCs from the quality controls ranged from 0.95 to 0.99 [23]. All the Bayley-III assessments were video-recorded for quality purposes. The Bayley-III raw scores were converted into scaled and composite scores based on U.S. citizen normative data [22]. For the analyses, we used raw, scaled, and composite scores.

Anthropometry

Weight was measured with a portable electronic scale (model 877, Seca, California, United States of America) that measures to the nearest 0.01 kg, and length was measured with portable length board (model 417, Seca, California, USA). We measured length and weight at the clinic or at home during the monthly follow-up visits. All anthropometric measurements were performed twice. The mean values were used in the analyses.

Laboratory procedures

Blood samples were collected from the cubital veins into polypropylene tubes containing EDTA (Sarstedt, Germany), which were protected from direct sunlight exposure. Up to 4-mL blood was collected at enrollment and end of study. The hemoglobin concentration was analyzed immediately following blood sampling with HemoCue (HemoCue 201, Ångelholm, Sweden), which was calibrated as per the guidelines defined by the manufacturer. The blood was centrifuged at room temperature for 10 min at 2,000 to 2,500 g within 10 min after venipuncture (Model R-304, Remi, Mumbai, India). Plasma and blood cells were separated, transferred into polypropylene vials (Eppendorf, Germany), and immediately stored at <−80˚C until analysis at Bevital Laboratory (Bergen, Norway; www.bevital.no). The samples were shipped to Norway on dry ice by World Courier. The plasma concentrations of cobalamin and folate were determined using microbiological assays [24,25] using a colistinsulfate-resistant strain of *Lactobacillus leichmannii* or chloramphenicol-resistant strain of *Lactobacillus casei*, respectively. The functional biomarkers plasma total tHcy and MMA are considered to be sensitive markers of B_{12} deficiency [26]. Plasma tHcy and MMA were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS) based on methylchloroformate derivatization [27]. The within-day coefficient of variation was 4% for both cobalamin and folate and ranged from 1% to 5% for tHcy and MMA. The between-day coefficient of variation was 5% for both cobalamin and folate and ranged from 1% to 8% for MMA and tHcy. We also calculated a combined indicator of cobalamin status (3cB12) based on the 3 biomarkers cobalamin, tHcy, and MMA as suggested by Fedosov and colleagues [28]. In short, this index is the log of the cobalamin concentration divided by the product of the log of the tHcy and MMA concentrations.

Sample size

The study had 80% power to detect a standardized effect size of 0.22 and had 90% power to detect an effect size of 0.28. In these calculations, we assumed a loss to follow-up of 10%. Details on the samples size calculations are also presented in the previously published protocol paper [18].

Statistical analyses

The analyses in this study were carried out according to the predefined protocols and analysis plans (S1–S4 Texts). Weight for age, weight for length, and length for age z-scores were calculated using the most recent WHO growth charts [29]. We defined underweight, stunting, and wasting as z-scores below −2 [29]. We depicted the relationship between changes in vitamin B_{12} status (i.e., change in the 3cB12 values from baseline to end of study) according to vitamin B_{12} status (3cB12) at baseline. To do so, we performed a kernel-weighted local polynomial regression of delta 3cB12 on baseline 3cB12 values by treatment group and depicted these dose-response graphs of the predicted values with 95% confidence intervals (CIs). We also compared the concentrations of cobalamin, tHcy, and MMA between the study groups in all infants and according to 3cB12 categories ("possible deficient," "low," and "adequate") at baseline. As these variables were left-skewed, we present the geometric means and the geometric standard deviation factors (GSDFs). We used the log-transformed values of the end-study biomarker concentrations when comparing the differences between the study groups. The exponentials of these mean differences are presented as the geometric mean ratio (GMR) between the study groups.

We compared the mean end-study Bayley-III scaled and composite scores between the intervention groups. For the other outcome variables (Bayley-III raw scores, growth, and hemoglobin concentrations), the effects of the intervention were estimated by comparing the changes from baseline to end study between the study groups. The precision of the effect estimates and corresponding *P* values were calculated using the Student *t* test assuming equal variances. We also analyzed the data by several predefined subgroups. The subgrouping variables were stunting (defined as length for age z-scores <-2), underweight (weight for age z-scores <-2), low vitamin B_{12} status (3cB12 <-0.5), low birth weight (birth weight <2,500 g), anemia (hemoglobin concentration <11.0 g/dL), and exclusive breastfeeding. For these analyses, we used univariate and multiple generalized linear models with the Gaussian distribution family and identity link function adjusting for a set of predefined potential confounders. The following variables were adjusted for in all subgroup analyses: length for age z-scores, maternal and paternal education, and age of the child at baseline. All analyses were performed using Stata version 16 (StataCorp, College Station, Texas, USA).

Results

From April 2015 to February 2017, we screened 733 infants and randomized 600 into the study (Fig 1). A total of 26 infants dropped out due to refusal or migration. Two children were unable to complete the end-study activities, leaving 572 infants with complete neurodevelopmental assessments. We were able to collect and analyze blood samples for biomarker assessments at end study from 567 children.

Baseline features by intervention and placebo groups are presented in Table 1. One in every five infants was born at low birth weight, and one-third were stunted (<−2 z-score length for age) at enrollment. The baseline features were evenly distributed between the intervention groups. The baseline status of vitamin B_{12} and its plasma biomarkers were also comparable between the intervention groups (S1 Table).

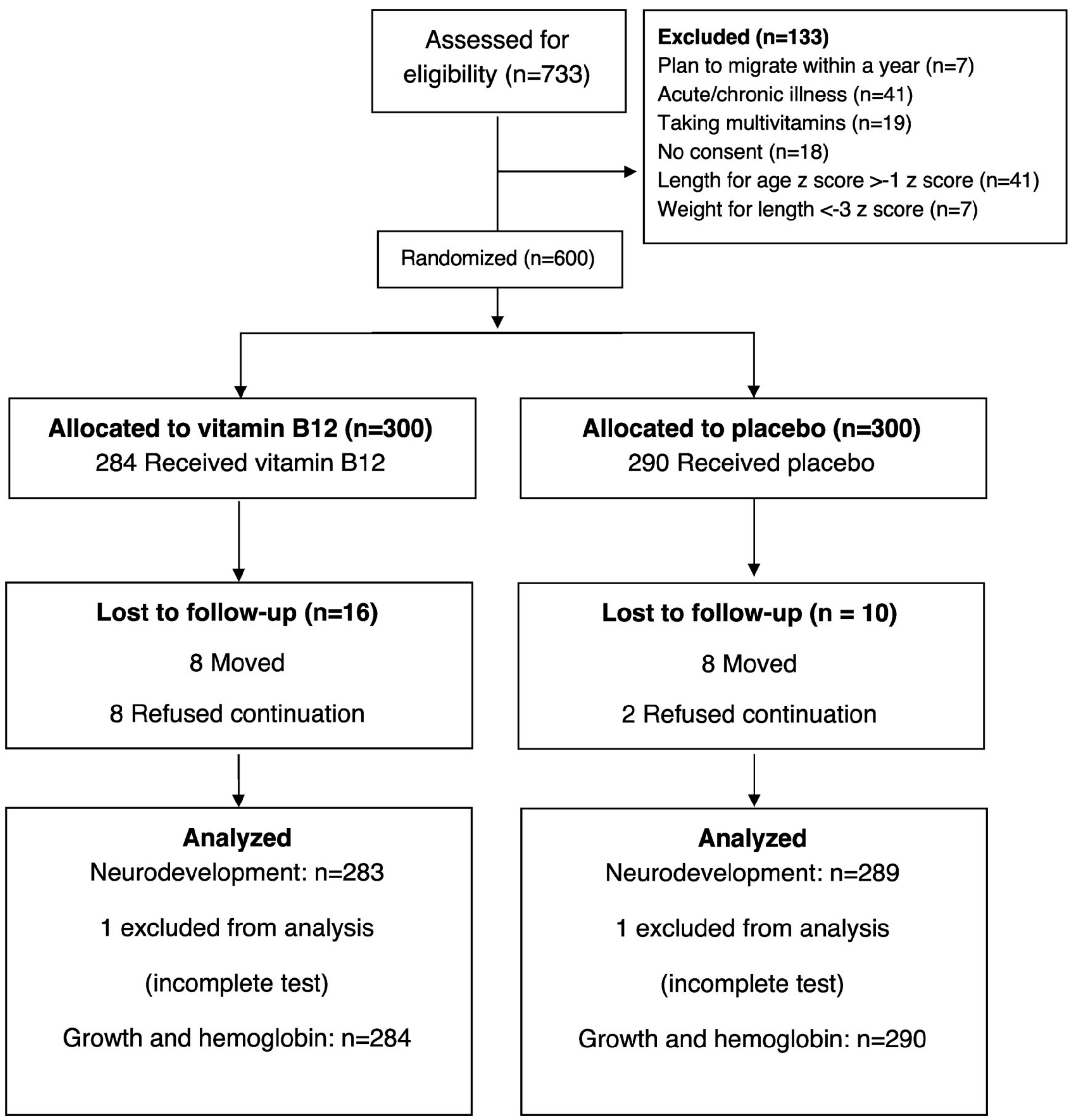

Fig 1. Trial flowchart of a study measuring the effect of daily vitamin B_{12} supplementation in Nepalese infants.

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Table 1. Baseline characteristics in a study investigating the effect of daily vitamin B_{12} supplementation on neurodevelopment and growth in 600 Nepalese infants.

	Vitamin B_{12} group (n = 300)		Placebo group (n = 300)	
	n	%	*n*	%
Infant characteristics				
Mean age of child (months), mean ± SD	8.1 ± 1.7		8.0 ± 1.8	
Male child	158	53	151	50
Have older siblings	155	52	153	51
Low birth weight (<2,500 gm)[1]	56	19	59	20
Hospitalization within first month of age	28	9.3	26	8.7
Demographic features				
Mother's age, mean ± SD	27.1 ± 4.7		27.5 ± 4.6	
Father's age[2], mean ± SD	30.0 ± 7.1		30.6 ± 5.1	
Mothers who completed secondary school or above	197	65.7	180	60
Fathers who completed secondary school or above	199	66.3	189	63
Mothers who work	117	39.0	110	36.7
Fathers who work	286	95.3	280	93.3
Socioeconomic status				
Family staying in joint family	143	47.7	149	49.7
Family residing in rented house	152	50.7	139	46.3
Number of rooms in use by the household (<−2)	163	54.3	174	58
Kitchen and bedroom in the same room	148	49.3	150	50
Family having own land	138	46	144	48
Receiving remittance from abroad	30	10	27	9
Breastfeeding status				
No breastfeeding at time of interview	8	2.7	6	2
Exclusive breastfeeding for 3 months or more	143	47.7	137	45.6
Nutritional status of infants				
Underweight (weight for age z-score <−2)	62	20.7	50	16.6
Stunting (length for age z-score <−2)	96	32.1	98	32.7
Wasting (weight for length z-score <−2)	12	4.0	7	2.3
Hemoglobin, g/dL, mean ± SD	10.6 ± 0.96		10.6 ± 0.91	
Anemia (hemoglobin <11 g/dL)	183	61	202	67.3
Nutritional status of mother				
BMI of mother, mean ± SD	23.7 ± 3.5		23.7 ± 3.6	
<18.5 kg/m^2 BMI of mother	15	5	19	6.3

[1]Among 579 infants whose birth weights were recorded.
[2]Among 487 fathers who were available.
n, number.

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Compliance

More than 94% of the prescribed doses were reportedly taken (94.3% in the vitamin B_{12} group and 94.0% in the placebo group; S2 Table). Of these, 86.3% and 84.7% of the vitamin B_{12} and placebo group, respectively, consumed the entire prescribed doses.

End points

Vitamin B_{12} supplementation had no effect on any of the neurodevelopmental outcomes (Table 2). For example, the cognitive composite scores were 0.73 points (95% CI: −0.55 to 2.02,

Table 2. Effect of daily vitamin B_{12} supplementation for 1 year starting in infancy on the Bayley Scales of Infant Development scores among infants in Bhaktapur, Nepal.

Bayley-III subscales	Vitamin B_{12} group (n = 283)	Placebo group (n = 289)	Mean differences
	Mean ± SD	Mean ± SD	(95% CI) (P value)
Change in raw scores from baseline until end study			
Cognitive	20.9 ± 4.3	20.7 ± 4.2	0.16 (−0.54 to 0.87) (0.648)
Language			
Expressive	14.4 ± 4.7	14.8 ± 5.0	0.35 (−0.44 to 1.14) (0.387)
Receptive	10.9 ± 4.0	11.2 ± 3.6	−0.31 (−0.94 to 0.32) (0.334)
Motor			
Fine motor	13.8 ± 3.3	13.7 ± 2.9	0.10 (−0.41 to 0.60) (0.712)
Gross motor	21.4 ± 5.1	21.7 ± 4.7	−0.28 (−1.08 to 0.52) (0.491)
Socio-emotional	29.4 ± 14.3	31.0 ± 12.0	−1.57 (−3.96 to 0.82) (0.197)
End-study composite and scaled score			
Cognitive composite score	90.5 ± 8.2	91.2 ± 7.3	0.73 (−0.55 to 2.02) (0.261)
Language composite score	93.0 ± 12.8	92.6 ± 12.6	−0.42 (−2.51 to 1.66) (0.692)
Expressive scaled score	8.6 ± 2.6	8.5 ± 2.4	−0.01 (−0.43 to 0.41) (0.965)
Receptive scaled score	9.0 ± 2.3	8.9 ± 2.5	−0.13 (−0.53 to 0.27) (0.517)
Motor composite score	99.9 ± 8.7	100.2 ± 8.3	0.32 (−1.08 to 1.73) (0.652)
Fine motor scaled score	10.7 ± 1.6	10.9 ± 1.8	0.22 (−0.06 to 0.50) (0.131)
Gross motor scaled score	9.2 ± 2.0	9.1 ± 1.7	−0.12 (−0.42 to 0.19) (0.457)
Socio-emotional composite score	104.3 ± 16.7	103.4 ± 17.1	−0.92 (−3.70 to 1.86) (0.518)

The mean differences, the corresponding 95% CI, and P values were calculated using Student t test assuming equal variances.

Bayley-III, Bayley Scales of Infant and Toddler Development 3rd ed.; CI, 95% confidence interval; n, number.

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P = 0.261) lower in the vitamin B_{12} group compared to the placebo group. There was also no effect on growth or hemoglobin concentration (Table 3). Children in both groups grew on an average 12.5 cm (SD: 1.8), and the mean difference was 0.20 cm (95% CI: −0.23 to 0.63, P = 0.354). The mean difference in hemoglobin concentration between the groups was 0.02 g/dL (95% CI: −1.33 to 1.37, P = 0.978). The adjusted effects of the interventions on growth and neurodevelopment by

Table 3. Effect of vitamin B_{12} supplementation on growth and hemoglobin concentrations among infants in Bhaktapur, Nepal.

	Vitamin B_{12} group (n = 283)	Placebo group (n = 290)	Mean differences
	Mean ± SD	Mean ± SD	(95% CI) (P value)
Change from baseline			
Length (cm)	12.5 ± 1.8	12.5 ± 1.8	0.09 (−0.21 to 0.39) (0.574)
Weight (kg)	2.1 ± 0.5	2.1 ± 0.6	−0.01 (−0.10 to 0.09) (0.919)
Hemoglobin (g/dL)	1.0 ± 1.1	1.0 ± 1.2	−0.02 (−0.20 to 0.17) (0.876)
End study			
Length (cm)	78.2 ± 2.6	78.4 ± 2.6	0.20 (−0.23 to 0.63) (0.354)
Weight (kg)	9.4 ± 0.9	9.4 ± 1.0	−0.02 (−0.18 to 0.13) (0.780)
Length for age z-score	−1.8 ± 0.7	−1.7 ± 0.7	0.05 (−0.06 to 0.16) (0.411)
Weight for height z-score	−0.7 ± 0.8	−0.8 ± 0.8	−0.08 (−0.22 to 0.05) (0.238)
Weight for length z-score	−1.4 ± 0.7	−1.4 ± 0.8	−0.04 (−0.16 to 0.08) (0.529)
Hemoglobin (g/dL)	11.6 ± 0.8	11.6 ± 1.0	0.02 (−1.33 to 1.37) (0.978)

The mean differences, the corresponding 95% CI, and P values were calculated using Student t test assuming equal variances.

CI, 95% confidence interval

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various subgroups are shown in S1 and S2 Figs. These analyses did not reveal any variable that modified the effect on any of the outcomes. Unadjusted subgroup analyses yielded the same results. We found no adverse effects of vitamin B_{12} supplementation (S3 Table).

The effect of vitamin B_{12} supplementation on B_{12} status expressed by the 3cB12 according to baseline status is depicted in Fig 2. The distance between the solid and dotted lines represents the metabolic effect of the intervention, which decreased as the baseline vitamin B_{12} status improved. A similar trend is displayed in S1 Table where the effects on the different biomarkers are shown. For the functional biomarkers, tHcy and MMA, poorer vitamin B_{12} status at baseline was associated with a larger effect of vitamin B_{12} supplementation. For example, when restricting the analyses to infants who were classified as possibly deficient, the placebo group had 60% higher MMA concentrations (indicating poorer vitamin B_{12} status) at end study compared to those in the vitamin B_{12} group (GMR 1.60, 95% CI: 1.20 to 2.14, $P < 0.001$). In the children who had adequate status at baseline, there was no effect of vitamin B_{12} supplementation on the MMA concentration (S1 Table).

Discussion

In this year-long, double-blind, placebo-controlled RCT, daily intake of a supplement containing vitamin B_{12} improved vitamin B_{12} status in marginally stunted Nepalese infants. Those with poor status at the onset of the study benefitted more than those with adequate status. The intervention, however, did not result in any improvements in neurodevelopment, nor was there any effects on growth or hemoglobin concentration. Restricting the analyses to those with poor status at baseline, i.e., those who also had the best metabolic response, did not alter these results.

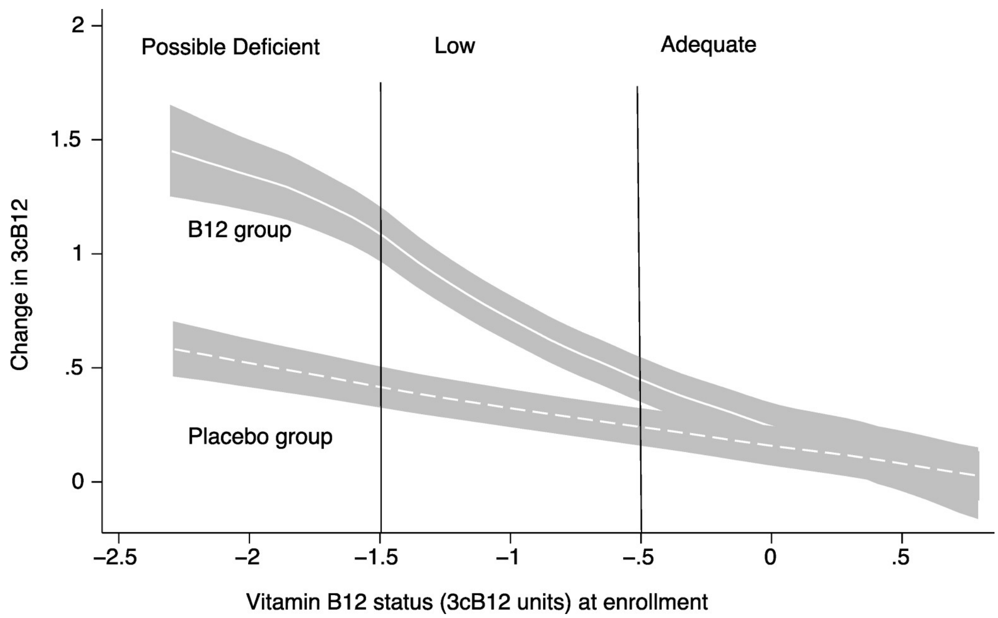

Fig 2. The association between vitamin B_{12} status at baseline and change in vitamin B_{12} status from baseline to end study by randomized group. The y-axis is the change in 3cB12 from baseline to end study, and the x-axis is the baseline 3cB12 value. The regression lines were generated by a kernel-weighted local polynomial regression with the change in 3cB12 as the dependent variable and 3cB12 at baseline as the independent variable. The shaded areas represent the 95% CI of the regression lines. The profiles were generated separately for the 2 intervention groups, and the distance between the 2 regression lines represents the effect of the vitamin B_{12} supplementation. The 3cB12 is a function of the plasma concentrations of cobalamin, tHcy, and MMA. CI, 95% confidence interval; MMA, methylmalonic acid; tHcy, total Homocysteine.

https://doi.org/10.1371/journal.pmed.1003430.g002

Our findings are in contrast to results from the observational studies where vitamin B_{12} status was positively associated with neurodevelopment, growth, and hemoglobin concentration [10,14,30]. The findings are also at odds with results from the 3 small (group sizes of 32 to 104) RCTs in infants and young children where vitamin B_{12} supplementation resulted in improved developmental scores [15–17].

Several elements of our study design and conduct support the veracity of our findings. The trial enrolled 600 children, of whom >95% could be included in the analyses. As a result, we have precise effect estimates of our primary outcomes, and thus, sufficient power to detect clinically meaningful differences between the treatment groups. The randomization was successful as indicated by no differences in baseline characteristics, and few participants were lost to follow-up. Finally, the compliance with supplement use was high, as it was given on nearly 95% of the scheduled days and resulted in an excellent metabolic response (S2 Table).

The study team has extensive experience with the Bayley-III, and the inter-rater reliability was excellent during both the initial standardization exercises as well as for the quality controls throughout the study. The Bayley-III scores were associated with established risk factors for poor neurodevelopment [23], which provided support for the validity of the test in this study setting. In addition, the study staff were trained and standardized for 2 decades in measuring infant growth, ensuring precise measurements for these outcomes. Lastly, we had an effective cold chain and used state-of-the-art biochemical methods to estimate the biomarker concentrations ensuring optimal description of the vitamin status.

The participants were moderately stunted infants and accordingly at risk of poor neurodevelopment and vitamin B_{12} deficiency. Thus, they constituted a group of children where we could expect an effect of vitamin B_{12} supplementation if subclinical deficiency affected any of our outcomes. For practical and ethical reasons, we could not target children with defined or overt B_{12} deficiency. We did not have the necessary diagnostic recourses to measure vitamin B_{12} status before randomization, and giving a placebo to infants with diagnosed vitamin B_{12} deficiency for a year would violate the principle of clinical equipoise. Thus, the study included some children with adequate vitamin B_{12} status at baseline. It is plausible that inclusion of vitamin B_{12} replete children could attenuate a potential effect of the intervention. However, this does not explain our null findings as the effect estimates did not change when we restricted the analyses to those with evidence of deficiency.

A higher dose or a different, more effective, mode of administration (i.e., injection) could have led to different results. The metabolic response, however, indicates a substantial biological effect. Vitamin B_{12} is involved in 2 biochemical reactions in humans [31]. In 1 of these, vitamin B_{12} is required for the transfer of methyl groups, which includes remethylation of homocysteine to methionine. Disruption of this pathway increases homocysteine and affects gene regulation and DNA synthesis [31]. Vitamin B_{12} also acts as an enzymatic cofactor for methylmalonyl-CoA mutase, an enzyme involved in the catabolism of fats and amino acids. Disruption of the latter pathway explains the increased MMA observed in vitamin B_{12} deficiency. Our study provides causal evidence that both of these metabolic pathways are affected by mild vitamin B_{12} deficiency in children, and their functioning improves with supplementation. Thus, despite no effects on the clinical outcomes, children in this population could benefit from increasing the vitamin B_{12} intake as indicated by their improved metabolic profile.

The period of supplementation in the present study might not have covered a critical window where adequate vitamin B_{12} status is crucial for optimal growth and neurodevelopment. Thus, initiating supplementation sooner, such as before or during pregnancy or earlier in infancy, could have yielded different results.

Restricting the participants to mildly stunted children increased the internal validity and statistical power of our study at the expense of the external validity. In other words, our recruitment strategy reduced the generalizability of our results, which is a limitation. It should be noted that enrolling all infants from the community would result in a larger proportion with adequate vitamin B_{12} status and, consequently, more children who would not respond to the intervention. A design ensuring higher external validity could accordingly attenuate a potential effect and, at the same time, increase the variability, both contributing to reduced statistical power. Reduced statistical power increases the risk of false-negative results (type II errors), which particularly question null findings such as here. In addition, our study was designed to measure the effects of vitamin B_{12} supplementation on several outcomes across several subgroups, which reduced the probability of overlooking relevant short-term clinical outcomes.

An important assumption of our study was that the inclusion criteria ensured we targeted those who would benefit from supplementation. It is possible, however, that these stunted children would suffer from deficiencies of other growth-limiting nutrients. We believe this potential bias was accounted for by providing other vitamins and minerals, by treating common infections, and by giving zinc for diarrhea.

In summary, our results show that in Nepalese infants, daily vitamin B_{12} supplementation improves vitamin B_{12} status and the metabolic profile expressed by the composite 3cB12 indicator. The metabolic response indicates that this population could benefit from vitamin B_{12} supplementation, but the clinical consequences of subclinical deficiency in early childhood remain uncertain.

Supporting information

S1 Fig. The effect of vitamin B_{12} supplementation on the Bayley Scales of Infant Development subscale scores in different subgroups. A point estimate to the left of the vertical line indicates a beneficial effect of vitamin B_{12}. None of the subgroup specific effects were statistically significant. The effect estimates were calculated with multiple general linear models with the Gaussian distribution family and identity link function adjusting for length for age z-scores, maternal and paternal education, and age of the child at baseline. Stunting and underweight were defined as being <-2 length for age z-scores and weight for age z-scores, respectively. 3cB12: combined vitamin B_{12} status indicator as suggested by Fedosov and colleagues [28], low 3cB12 is <-0.5, low birth weight: birth weight <2,500 g, anemia: hemoglobin concentration <11 g/dL.
(TIF)

S2 Fig. The effect of vitamin B_{12} supplementation on growth and hemoglobin concentration in different subgroups. A point estimate to the left of the vertical line indicates a beneficial effect of vitamin B_{12}. None of the subgroup specific estimates were statistically significant. The effect estimates were calculated with multiple general linear models with the Gaussian distribution family and identity link function adjusting for length for age z-scores, maternal and paternal education, and age of the child at baseline. Stunting and underweight were defined as being <-2 length for age z-scores and weight for age z-scores, respectively. 3cB12: combined vitamin B_{12} status indicator as suggested by Fedosov and colleagues [28], low 3cB12 is <-0.5, low birth weight: birth weight <2,500 g, anemia: hemoglobin concentration <11 g/dL.
(TIF)

S1 Table. Effects of daily vitamin B_{12} supplementation for 1 year starting in infancy on markers of vitamin B_{12} status.
(DOCX)

S2 Table. Compliance of vitamin B_{12} supplementation among Nepalese infants participating in clinical trial on the effect of vitamin B_{12} supplementation on growth, development, and hemoglobin concentration.
(DOCX)

S3 Table. Adverse effects of vitamin B_{12} supplementation among Nepalese infants participating in clinical trial on the effect of vitamin B_{12} supplementation on growth, development, and hemoglobin concentration.
(DOCX)

S1 Text. Main protocol version 1.0. August 1, 2014.
(DOCX)

S2 Text. Main protocol version 2.1. October 16, 2016.
(DOCX)

S3 Text. Plan of analysis version 1. March 2018.
(DOCX)

S4 Text. Plan of analysis version 2.1. October 2019.
(DOCX)

S1 Checklist. CONSORT Checklist.
(DOCX)

Acknowledgments

We would like to express our gratitude to all the field staff, children, and families in Bhaktapur who participated in the study. We are also grateful to the Child Health Research Project Team at the Department of Child Health at the Institute of Medicine, Tribhuvan University and Siddhi Memorial Foundation and its founder Shyam Dhaubhadel. Finally, we thank Johanne Haugen who was responsible for randomization.

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Government of Nepal
Nepal Health Research Council (NHRC)

Ref. No.: 1056

16 December 2016

Dr. Ram Krishna Chandyo
Dr. Laxman Prasad Shrestha
Principal Investigator
Child Health Research Project, Institute of Medicine
Kathmandu, Nepal

Subject: Approval of research proposal entitled **Supplementation of Vitamin B12 in pregnancy and postpartum on growth and neuro development in early childhood: A Randomized, placebo Controlled Trial among Pregnant women in Bhaktapur, Nepal**

Dear Dr. Chandyo and Dr. Shrestha
It is my pleasure to inform you that the above-mentioned proposal submitted on **31 August, 2016** (**Reg.no. 253/2016** please use this Reg. No. during further correspondence) has been approved by NHRC Ethical Review Board on **07 December 2016**.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol before the expiration date of this approval. Expiration date of this study is **December 2019.**

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their research proposal and submit progress report and full or summary report upon completion.

As per your research proposal, the total research amount is **NRs. USD 500,000.00** and accordingly the processing fee amount to **NRs. 1,613,550.00.** It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E section of NHRC.

Thanking you,

....

Dr. Khem Bahadur Karki
Member Secretary

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.org.np, E-mail: nhrc@nhrc.org.np

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			04.01.2017	0000

Vår referanse må oppgis ved alle henvendelser

Tor Strand
Forskningsavdelingen

2016/1620 Effekten av vitamin B12-tilskudd hos Nepalske gravide på barnas vekst og utvikling

With reference to your appeal regarding the abovementioneded project.

Institution responsible for the research: University in Bergen
Project manager: Tor Strand

Background
The project was reviewed by REC Western Norway in the meeting 27.10. REC Western Norway decided we need more information about:
1. The need for yet another study on vitamin B12.
2. Is it ethically acceptable to conduct a placebo controlled study, taken into consideration the scientific knowledge we already have on vitamin B12 and the fact that we must assume that many in the study population lack vitamin B12? Why not find a study design where all participants have vitamin B12?

REC Western Norway has also received an assessment from an external expert on these questions. The project manager has been asked to address the remarks in this report.

New assessment 24.11.2016
The Comittee decided to reject the application based on the following points:
1. The problematic placebo arm.
2. Revealing deficiencies in the pregnant women and not treating them.
3. Current evidence.
4. Inconsistencies between the application and the response

The project manager appealed the decicion and the appeal was assessed in the meeting 12.01.2017.

New factors in the appeal
The project manager again points to that there is insufficient current evidence and that the study is deemed necessary. There is attached a recommendation about supplementation for pregnant women from the WHO, where vitamin B12 is not included in the recommendation. The project manager points out that measurements taken at baseline will be analyzed in Norway, and will not be available on the premises until after a longer period.

Furthermore the local approval by the Ethics Committee in Nepal has been obtained.

Finally based on discussion raised by the ethics committee the research group suggests some changes in the project:
- There will be an early measurement of neuronal development at interim analysis at 100 patients enrolled

- Women planning to take B12 supplement will be excluded
- If the authorities change their recommendations on intake, the treatment will be given all.
- Where the study doctor finds it necessary to treat the woman with vitamin B12 or vitamin B12 containing supplements, the women will be excluded from the study and given treatment.

The Committee Chairman also chose to retrieve a second independent expert opinion, that was presented for the Committee.

New assessment 12.01.2017
Based on the changes and clarifications that have been made, the Committee chooses to approve the project.

The Committee considers it crucial that women who are considered to need treatment with B12 by the study doctor will receive this, and get excluded from the study.

The Committee emphasizes that all women participating will benefit from participation and that the safety monitoring presented is detailed and thorough. The Committee also emphasizes that WHO's recommendation does not include B12 and that there may be sufficient doubt about current evidence so that a placebo controlled trial can by ethically justified.

Vedtak
REC Western Norway accepts the appeal and approves the project.

Sincerly,

Marit Grønning
Prof. dr.med
Chairman

Øyvind Straume
Committee Secretary

Kopi til: postmottak@uib.no

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May 25, 2021

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