

Vitamin B12 Deficiency in Pregnancy and Lactation: Is there a Need for Pre and Post-natal Supplementation?

Towfida J Siddiqua¹, Lindsay H Allen^{2,3}, Rubhana Raqib¹ and Tahmeed Ahmed¹

¹International Centre for Diarrheal Disease Research, Bangladesh

²USDA, ARS Western Human Nutrition Research Center, Davis, CA, USA

³Department of Nutrition, University of California, Davis, CA, USA

*Corresponding author: Tahmeed Ahmed, Director and Senior Scientist, Centre for Nutrition and Food Security, icddr, b 68 Shaheed Tajuddin Ahmed Sarani Mohakhali, Dhaka-1212, Bangladesh, Tel: +880-2-9827103; Fax: +880-2-9827101; E-mail: tahmeed@icddr.org

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Abstract

This article reviews vitamin B12 (B12) status and deficiency during pregnancy and lactation, its effect on pregnancy, and the health of the offspring, with the aim to underscore the need for a sustainable strategy to improve maternal and infant vitamin B12 status of low and middle income countries. Vitamin B12 is a basic nutrient required for maintenance of normal erythropoiesis, cell reproduction, nucleoprotein and myelin synthesis. B12 deficiency is associated with adverse pregnancy outcomes and neurodevelopmental morbidity during infancy. Very few studies have indicated that B12 deficiency may contribute to altered immune responses in animals and humans. Additionally, no studies have shown modulation of infant motor development in response to maternal B12 supplementation. Despite the high global prevalence of B12 deficiency and its serious effects on pregnant women and offspring, there is still no consensus on the cut-off of biochemical markers (indicator of B12 deficiency) to correctly diagnose B12 deficiency in mother-infant dyad. Also, the optimum dose of B12 to normalize B12 status of mother-infant pairs in a deficient population is not known yet. In addition, markers of other functions such as neurodevelopment, immune response that may be affected by vitamin B12 deficiency should be measured to determine if they respond to supplementation. Thus, there is an urgent need to conduct more trials to find out the optimum dose, to investigate whether intervention with such pre-and post natal vitamin B12 supplementation would improve maternal, neonatal and child health outcomes in population at risk, giving emphasis on neurological processes, immune functions and epigenetic modifications. Other strategies including food based approach also require evidence based results which will help to understand effectiveness of a targeted and well-designed intervention among this population.

Keywords: Vitamin B12; Pregnancy; Lactation; Infancy

Introduction

Vitamin B12 (Cyanocobalamin), a water-soluble vitamin is an essential nutrient required for maintenance of normal erythropoiesis, nucleoprotein and myelin synthesis, cell reproduction and normal growth. Vitamin B12 is a necessary cofactor in the methionine synthase reaction, which converts homocysteine into methionine. Deficiency of vitamin B12 could elevate plasma homocysteine which is a risk factor for cardiovascular diseases. Methionine is the required precursor for the formation of S-adenosylmethionine, a universal methyl donor essential for methylation of phospholipids, neurotransmitters, amines, DNA, RNA and myelin basic protein. A reduction in this important methyl donor causes impaired DNA methylation that may contribute to altered fetal metabolic programming and increased risk for chronic diseases later in life. In the mitochondria vitamin B12 is required as a cofactor for the conversion of methylmalonyl-CoA to succinyl-CoA by the enzyme methylmalonyl-CoA mutase. In B12 deficiency, the concentration of methylmalonyl CoA is elevated and forms the by-product methylmalonic acid (MMA). Thus vitamin B12 insufficiency may influence carbohydrate and lipid metabolic pathways [1].

Emerging data suggest that deficiency of vitamin B12 [higher plasma B12 and holotranscobalamin (holoTC); lower plasma

methylmalonic acid (MMA) and total homocysteine (tHcy) concentrations] is highly prevalent in pregnant and breastfeeding women and their infant. Impaired vitamin B12 status during pregnancy is associated with increased risk of birth defects and common complications [e.g. intrauterine growth restriction, preterm delivery, neural tube defects (NTD)] [2], and possibly immune function impairment [3,4]. Most of the case studies indicate that maternal depletion of the vitamin is the predominant reason for deficiency in the infant [5]. Vitamin B12 deficiency in early life may result in short- and long-term effects on infants' neurological and cognitive functions which will have profound effects on health, development and achievement of full human capacity of an entire generation of children. Maternal supplementation with B12 from early pregnancy through lactation might be an effective approach to improve both maternal and infant status, by increasing stores in utero and concentrations in breast milk. This review summarizes literature relating to B12 status and deficiency during pregnancy and lactation, its effect on pregnancy, and the health of the offspring, with the aim to underscore the need for a sustainable strategy to improve maternal and infant vitamin B12 status of low and middle income countries.

Methods and Materials

A comprehensive literature review was performed to identify articles describing the association of vitamin B12 deficiency with

adverse pregnancy outcomes and interventions with B12 during pregnancy and infancy. Databases searched included PubMed and Google Scholar. Initial key words for the searches included “vitamin B12”, “pregnancy”, “lactation”, “fetus or neonate”, “infant” and “supplementation or intervention”. Outcome measures searched included hematology, birthweight, growth, fetal growth retardation, immune response, DNA methylation and neurodevelopment. These terms were also used to undertake searches using the term “cobalamin”. Further studies were identified by searching for additional terms based on results obtained from the initial searches in PubMed. Articles reporting on non-pregnant women, elderly populations and not reporting outcome of interest were screened out. Based on titles and abstract over 2000 articles were identified.

Presented here is the brief discussion on homeostasis and requirements of vitamin B12 during pregnancy and lactation. This is followed by a discussion on deficiency of vitamin B12, its adverse pregnancy outcomes and currently available studies on intervention with vitamin B12 during pregnancy and infancy.

Results

Vitamin B12 homeostasis and requirements during pregnancy and lactation

The demand for vitamin B12 increases during pregnancy due to rapid cell multiplication resulting from the uterine enlargement, placental development, and fetal growth [6]. Animal and human studies suggest that absorption of the vitamin may become more efficient during pregnancy [7,8]. The number of receptors is usually the rate limiting factor determining the amount of B12 absorbed via the ileal receptors. Placental lactogen acts as a regulator of increased Intrinsic Factor (IF)-mediated vitamin B12 binding to ileal binding sites by recruiting already existing cryptic receptor rather than stimulating the synthesis of new one [9]. However despite the increased efficiency of absorption total plasma B12 declines steadily throughout pregnancy commencing with the first trimester [10]. This gradual, physiologically normal decline in the plasma B12 is thought to be due to several factors such as hemodilution, hormone fluctuations, impaired renal function, or altered concentration of binding proteins (transcobalamin and haptocorrin) [11]. The lowest concentration is observed during third trimester and it returns to prepregnancy levels within a few weeks postpartum. Vitamin B12 is actively transported to the fetus, which has a significant influence for the progressive decline of maternal vitamin B12 levels during pregnancy. Fetal demand for the vitamin has been estimated to approximately 0.3 µg/day. Earlier studies by Luhby et al. showed that newly absorbed maternal B12 is more readily transported to placenta than maternal liver stores [12]. The fetal liver store of vitamin B12 is only 30% of the adult liver B12 content. Available evidence suggests that the fetus retains most of the plasma B12 to utilize for cellular reactions. The well-nourished human adult has about 2-5 mg of the vitamin, the majority being stored in the liver which is adequate without repletion for 3-5 years. A healthy pre-pregnancy body stores of B12 are, therefore, sufficient to meet increased demand during pregnancy [13].

Vitamin B12 secretion into breast milk is highly dependent on current maternal intake and absorption [14,15]. Human milk may contain 100-fold more haptocorrin (a vitamin B12-binding protein) than serum, mostly in its free form [16]. Due to higher haptocorrin, the B12-binding capacity of milk is 1000 times greater than plasma. Almost all B12 in breast milk is bound to haptocorrin that is stable to

proteolytic enzymes in the gastrointestinal tract. The function of high amounts of breast milk haptocorrin is unknown. A few studies proposed that the excess haptocorrin may play a host-defense function against pathogens in the gastrointestinal tract of breastfed infants [17,18]. However, this hypothesis was not supported by a systematic study on a panel of 34 commensal and pathogenic bacteria of infants [19]. There are few reports describing longitudinal changes in the concentration of breast milk vitamin B12. Samson et al. reported that the mean vitamin B12 binding capacity of colostrum is three times higher than that of mature milk [20]. Breast milk B12 concentrations decline from the high levels in colostrum to lower levels in mature milk [21]. Based on the amount estimated to maintain normal serum B12 concentrations and normal hematological status in half of the adult population, the estimated average requirement for adults (EAR) is set at 2.2 µg/day. The recommended dietary allowance (RDA) is 2.4 µg/day (an amount adequate to meet the requirements of 97.5% of healthy individuals). The rate of fetal accumulation (~0.1 to 0.2 µg/day throughout gestation) coupled with the increased efficiency of maternal absorption increases the EAR of vitamin B12 for pregnancy by 0.2 µg/day, with no distinction made for age of the mother. Therefore, the RDA is increased to 2.6 µg/d in pregnancy to support daily transfer to the fetus [22]. The Adequate Intake (AI) for infants (0 to 6 mo) is set at 0.4 µg/day. During the first 6 months of lactation, based on the few available data the average amount of B12 secreted in the milk of mothers with adequate B12 status is approximately 0.33 µg/day. To estimate the EAR for lactation, 0.33 µg/day of B12 is added to the EAR of 2 µg/day for adolescent girls and adult women; the result is rounded up to +2.4 µg/day. Because information is not available on the standard deviation of the requirement for B12, the RDA is set at 120 percent of the EAR. Thus the RDA for lactating women is 2.8 µg/day to replace secretion of the vitamin in breast milk [6].

Vitamin B12 deficiency

The first sign of vitamin B12 deficiency is characterized by a decrease in serum holoTC, after which both serum MMA and plasma tHcy start to increase, and finally there is a reduction in serum vitamin B12. The next stages of negative B12 balance is impaired erythropoiesis, accompanied by yet lower concentrations of holoTC and serum B12, and hypersegmented neutrophils. In the end hemoglobin concentrations are reduced which results in a macrocytic anemia [23]. The most severe form of manifestation of deficiency is sub-acute combined degeneration of the spinal cord, characterized by degeneration of the posterior and lateral columns of the cord [24].

Measurement of the total vitamin B12 concentration in plasma is the usual method for assessing vitamin B12 deficiency, despite limited specificity and controversy about sensitivity [25]. However, the plasma vitamin B12 concentration is not a reliable indicator of vitamin B12 status in pregnancy. Elevated MMA and tHcy are generally considered more sensitive for diagnosing deficiency than serum B12. However, MMA is affected by intestinal bacterial overgrowth and tHcy is elevated by deficiencies of vitamin B6, folate, and riboflavin. Recent investigations show that holoTC (the metabolically active fraction of B12 available to cells) is a more sensitive indicator of vitamin B12 status than the total serum vitamin B12 level or the serum concentration of MMA and plasma tHcys [26-30]. The reference values for non-pregnant women are often applied to assess B12 deficiency in pregnant women. Since the concentrations of vitamin B12, tHcy and MMA are known to decrease during the normal course of pregnancy, low concentrations of B12 and its metabolites later in pregnancy must be interpreted with caution. Recently Fedosov

proposed a new B12 status parameter (w) that combines all four of these biomarkers in a mathematical model [31]. Evidence is accumulating that this model is useful to make diagnosis of B12-related disorders unambiguous.

Dietary deficiency of vitamin B12 is a major problem in the Indian sub-continent, Africa, Central and South America and Mexico, where it is prevalent across the life span [32,33]. Emerging data suggest that deficiency of vitamin B12 (indicated by serum or plasma B12 <150 pmol/L) is highly prevalent in women of reproductive age, particularly amongst populations with limited intake of animal source foods. In addition to increased requirement during pregnancy and lactation, insufficient consumption of animal-source foods, malabsorption associated with gastric diseases and nonspecific gastritis further contribute to B12 deficiency [34]. It is difficult to quantify the prevalence of deficiency in pregnant women partly due to the gradual decline in the plasma B12 concentration throughout gestation. Based on gestational week prevalence of deficiency worldwide may vary from 5% (<28 days gestation) to 72% (immediately prior to delivery) [35,36]. In south Asia, examples of reported prevalence of deficiency include 27% of pregnant woman at early to late pregnancy (i.e. gestational week, GW 10.2 ± 4.1 to 32.6 ± 3.9) in rural Nepal, 74% in Haryana, 65% at GW 18-28 in Pune and 51% at GW ≤14 in urban south India [37-40]. The MINIMat study in Matlab, a rural area in Bangladesh showed that B12 deficiency was 46% in pregnant women at third trimester [41]. The JiViTA study in rural north-western Bangladesh found a prevalence of 20% deficiency (<150 pmol/L) in early pregnancy [42]. The newborns and infants of vitamin B12-deficient mothers have low B12 stores at birth which is further aggravated by very low availability of B12 in breast milk hindering their growth and development [43]. Maternal status of the vitamin prior to and during pregnancy, stores at birth and the concentration in breast milk all have an impact on infant B12 status [44,45]. This is substantiated by a study on maternal-newborn paired plasma samples (n=173) in Norway which shows that infants born to healthy, non-vegetarian mothers and particularly those who were breastfed, had poor B12 status. Importantly, all three markers of maternal impaired B12 status predicted low serum B12, high plasma MMA, and high plasma tHcy in the newborns [46].

Adverse pregnancy outcomes

Vitamin B12 plays a key role in normal functioning of brain and nervous system [47]. Throughout the lifecycle the most serious consequence of B12 deficiency is impaired development and function of neurological processes. The underlying mechanism may involve impaired myelination or demyelination; altered S-adenosylmethionine: S-adenosylhomocysteine ratio; imbalance of Tumor Necrosis Factor-alpha (TNF-alpha) and Epidermal Growth Factor (EGF) levels; and accumulated lactate in brain cells [48]. A study in Brazil showed that the S-adenosyl methionine (SAM): S-adenosyl homocysteine (SAH) ratio was significantly decreased in both B12-deficient pregnant women (GW: 37-42 wk) and their newborns. Lower maternal vitamin B12 concentrations (Geometric mean: 130 pmol/L) were associated with higher tHcy and lower SAM:SAH in newborns suggesting that methylation could be impaired in mother-infant pairs [49]. Schorah et al. found an association between low maternal plasma vitamin B12 and pregnancies affected by anencephaly [50]. Several studies reported that low maternal serum vitamin B12 is an independent risk factor for neural tube defects (NTDs) [51,52]. Reduced B12 binding by TC-II or holoTC also increases the risk of NTDs [53]. Nine years post-folic acid fortification,

a population based case-control study in Ontario, Canada reported almost a tripling in the risk for NTDs in the presence of low maternal B12 status, measured by serum holoTC [35]. A multicenter case-control study in India (n=318 cases and n=702 controls) demonstrated that mothers of NTD fetuses had higher plasma tHcy and lower holoTC concentrations and that a polymorphism in transcobalamin (TCN2, 776C>G) genes was a strong predictor of NTD. This study suggests a potential role of poor B12 status of Indian women in the etiology of NTD [54]. In general deficiency of vitamin B12 has been linked to a variety of abnormal neurological symptoms including: hypotonic muscles, failure to thrive, cerebral atrophy and developmental regression [48]. Maternal plasma B12 in pregnancy is also predictive of offspring cognitive performance at 9 years [55]. Children of mothers with low plasma B12 (lowest decile, <77 pmol/L) during pregnancy performed less well on tests of sustained attention and short-term memory compared to the children of mothers with higher plasma vitamin B12 (highest decile, >224 pmol/L). In rural Kenyan women (n=138), B12 intake during pregnancy was correlated with improved scores on the infant's Brazelton Neonatal Behavioral Assessment reflex subscale score (R = -0.19, p = 0.05; with adjustment for gestational age) within 3 days after birth [56]. An observational study in North Indian children (12-18 mo) demonstrated positive associations between infants' mental development index score and vitamin B12 status [57].

A number of studies have reported an association of vitamin B12 deficiency with infertility, IUGR, preeclampsia and early pregnancy loss [58-63]. One study in Chinese women found that inadequate preconception vitamin B12 (<258 pmol/L) was associated with a 60% increased risk of preterm delivery [64]. A cohort study in Bangalore, India, (n=486) showed that women in the lowest tertile of serum vitamin B12 concentration during each of the three trimesters of pregnancy had a significantly higher risk of IUGR [65]. A similar study carried out in South India (n=1838) observed that high folate and low vitamin B12 intakes (1.2 µg/d) during pregnancy are associated with small-for-gestational age infants [66]. Although there is little information linking vitamin B12 status with gestational diabetes, an observational cohort study (n=785) carried out in Mysore, India found an interesting association of B12 deficiency during pregnancy with obesity and gestational diabetes [67].

Increasing evidence suggest that folic acid supplemented pregnant women who may be in negative B12 balance, have increased risk for adverse maternal and infant outcomes (such as increased cardiometabolic disease risk). Low maternal B12 and a normal-to-high range of folate during pregnancy was associated with high insulin resistance and adiposity in the offspring at 5 years of age [39]. In rural Nepal, maternal low plasma B12 status in early pregnancy was associated with a significant elevation of HOMA-IR (Homeostatic model assessment-Insulin Resistance) in the 5 year old child [68]. These studies raised concerns that folic acid supplementation or added folate in the fortified foods may have adverse effects on women who have low vitamin B12 and high folate status during pregnancy or on the health of her offspring [69,70].

DNA methylation, a well characterized epigenetic mechanism, is essential for normal development and can be directly affected by dietary methyl donors (protein, folate, choline, methionine, vitamins B6 and B12) in the one-carbon metabolic pathway [71]. Evidence is accumulating that adequate maternal -fetal vitamin B12 status during pregnancy is critical for donating methyl groups for CpG methylation and epigenetic regulation [72,73]. Impaired DNA methylation might

ultimately affect infant development and predispose to higher disease risk later in life via developmental programming [74]. Also evidence from animal studies substantiates the importance of DNA methylation in influencing the phenotype of a growing fetus [75]. More recently a body of research has evolved around the imbalance in neurotrophic and neurotoxic cytokine levels as a key point in the pathogenesis of B12-deficient neuropathy [76].

Increasing evidence also suggests that vitamin B12 has important immunomodulatory effects on B- and T- cell function and humoral immunity [77-80]. In a study of Japanese men and women aged 36-83 y, B12 deficient (n=11) and control (n=13) subjects were injected with methyl-B12 every other day for two weeks to examine the effect on lymphocyte subpopulations and NK cell activity. Antibody-dependent cell-mediated cytotoxicity, lectin-stimulated lymphocyte blast formation, and serum immunoglobulin concentrations were not changed by methyl-B12 treatment [80]. However, vitamin B12 deficient subjects had markedly fewer and less active immune cells which were revived by restoring B12 status. A similar study in Turkey [81] reported that in pernicious anemia, abnormalities (lower CD8+ lymphocytes; higher CD4/CD8 ratio, and depressed NK cell activity) in the immune system are restored by vitamin B12 replacement therapy. Interestingly, concentrations of immunoglobulins (IgG, IgA and IgM) and complements (C3) were also elevated after cyanocobalamin treatment. These results suggest that vitamin B12 may act as a modulatory agent for cellular immunity, especially affecting CD8+ cells and the natural killer cell system. In a controlled, prospective cohort study [82] serum antibody titers to 12 pneumococcal serotypes were measured by radioimmunoassay before and 4 weeks after vaccination in patients (n=15) with low B12 serum

concentrations vs. controls (n=15) with normal B12 concentrations. Immunocompetent elderly subjects with low B12 concentrations had impaired antibody response to pneumococcal polysaccharide vaccine compared to the patients with normal B12. In addition, vitamin B12 remained an independent predictor of antibody response (when adjusted for mean corpuscular volume and age). These data may point towards beneficial effects of vitamin B12 on the humoral immune system and possibly the development of vaccine specific immunity. However there is a scarcity of information in demonstrating the consequences of maternal B12 deficiency on measures of maternal and infants' immunological function.

Interventions during pregnancy and infancy

There are not many reports on status and effects of pre- and postnatal vitamin B12 supplementation among mother-infant pair (Table 1). One of the earliest supplementation trials [7] with pregnant (n=31) and non-pregnant women (n=29) stratified the participants at baseline into three supplementation groups, 250, 500 and 1000 µg B12/d (oral dose). Serum B12 concentrations were measured one and one-half and three hours later. None of the women in either the pregnant or non-pregnant group showed any response to the 250 µg supplement. The pregnant women in the 500 µg supplemented group had a 41.5% increase in serum B12 vs. a 16.6% increase in non-pregnant women. Pregnant women supplemented with 1000 µg had an 88.8% increase in serum B12 while non-pregnant women had a 28.5% increase. These data suggests that both groups had a significant response to supplementation but the pregnant women showed greater vitamin B12 absorption than the non-pregnant women due to increased efficiency in absorption during pregnancy.

Study design	Results	Conclusion and Limitation
Hellegers A et al. [7]		
Pregnant (n=31) and non-pregnant women (n=29), were stratified into three supplementation groups, 250, 500 and 1000 µg B12/d (oral dose). Serum B12 concentrations were measured 1, 1.5 and 3 h later. Responder was defined as >160 µg increase observed in serum B12 concentration.	None of the women in either the pregnant or non-pregnant group showed any response to the 250 µg supplement. Pregnant women supplemented with 1000 µg had an 88.8% increase in serum B12 while non-pregnant women had a 28.5% increase.	Compared to non –pregnant women absorption of vitamin B12 is significantly increased in pregnancy. Serum B12 was the only response measured.
Eneroeth H et al. [83]		
RCT in Bangladesh, n=4436 (GW ≥14). Daily micronutrient supplements either: 1) folic acid and 30 mg iron; Fe30Fol or 2) folic acid and 60 mg iron; or 3) a multiple micronutrient including folic acid and 30 mg iron (MMS). Supplementation continued up to 3 mo postpartum.	46% (n=670) of women had low B12 status, At 6 mo prevalence of infant B12 deficiency significantly lower in the MMS group than in the Fe30Fol group (26.1 vs. 36.5%) .	B12 deficiency highly prevalent in this population; MMS may have a beneficial effect on B12 status in infancy. Small effect size; B12 was the only response measured; No assay of newborn B12 status.
Baylin A et al. [84]		
RCT in Tanzania. HIV-infected mothers (n=716); daily oral dose of one of four regimens: vitamin A, multivitamins (B including 50 µg B12, C, E), multivitamins including A, or placebo. Supplementation started at first prenatal visit and continued up to 6 mo postpartum.	Compared to infants from non-multivitamin-supplemented mothers, multivitamins increased B12 at 6 wk and 6 mo (mean differences=176 pmol/L, and 127 pmol/L, respectively), significant reductions in the prevalence of B12 deficiency at 6 mo.	Multivitamin (B, C, E) supplementation had major effect on serum B12 at 6 wk that was sustained through 6 mo of age. B12 was the only response measured; no information on breast feeding frequency; complementary feeding.
Duggan C [85]		
RCT in India. Pregnant women (<14 GW) randomized to receive either an oral B12 (50 µg/day) or placebo through 6 wk postpartum.	Compared to the placebo group, supplemented women had higher median plasma B12 concentrations at both the 2nd (216 vs. 111 pmol/L)	Oral maternal vitamin B12 supplementation is effective to improve maternal and infant B12 status.

	and 3rd (184 vs. 105 pmol/L) trimesters; higher breast milk B12 (136 vs. 87 pmol/L) in the placebo group; higher infant plasma B12 (199 vs. 136 pmol/L); reduced infant MMA and tHcy	HoloTC was not assayed; data were not available for women after postpartum; too short follow-up period for infants B12 status.
Monsen et al. [86]		
RCT in Norway. n=107 infants (6 wk) randomized to receive either an intramuscular injection of B12 (400 µg) or no intervention (control). Biomarkers were assayed at enrollment and at age 4 mo.	Compared to the control group supplement-treated infants had 75% higher median serum B12, raised serum B12 (IQR: 291–497 pmol/L), and lowered MMA (from 0.58 to 0.20 µmol/L) and tHcy (from 7.46 to 4.57 µmol/L) at 4 mo.	Supplementation can normalize a metabolic profile consistent with impaired B12 status in young infants. Control group did not receive placebo medication; effect on neurological parameters were not assessed.
Torsvik et al. [87]		
RCT, infants (n=79, <8 mo, plasma tHcy concentration ≥6.5 µmol/L) randomized to either an intramuscular injection of B12 (400 µg) or a sham injection	Supplementation decreased plasma tHcy by 54%, and MMA by 84%, no significant changes in the placebo group. Sig. higher motor function [Alberta Infants Motor Scale (AIMS)] score in the B12 group than in the placebo group (7.0 (5.0, 9.0) vs. 4.5 (3.3, 6.0)]. Higher proportion showed improvements in regurgitations (69% vs. 29%, respectively; P=0.003).	In infants with impaired B12 function, 400 µg intramuscular injection of B12 resulted in biochemical evidence of repletion and improvement in motor function and regurgitations; too short follow-up period (1 mo).
¹ RCT indicates randomized controlled trial.		

Table 1: Summary of interventions with vitamin B12 (pregnant/non-pregnant women and infants)¹

Many intervention studies among mother-infant dyads have included B12 in multivitamin supplements. The MINIMAT trial in Matlab reported multiple micronutrient supplementation with the recommended dietary allowances (RDA) (2.6 µg/d B12 in pregnancy up to 3 months postpartum) did not significantly impact on maternal B12 deficiency. However it only reduced infant deficiency at 6 mo to 26% [83]. In a randomized, placebo-controlled trial in Tanzania, multivitamin supplementation (with 50 µg B12) of HIV-infected mothers (n=716) throughout pregnancy upto 6 months postpartum significantly increased infant plasma vitamin B12 concentrations at age 6 weeks and 6 months (mean differences:176 and 127 pmol/L, respectively) and decreased the prevalence of vitamin B12 deficiency compared to the placebo [84]. However, adverse or improved outcomes cannot be attributed to any specific vitamin in such trials with multivitamin supplementation. A recent randomized study among Indian women reported that supplementation with B12 (50 µg/day) throughout pregnancy up to 6 wk postpartum increased the concentration in maternal and infant plasma and breast milk B12 content [85]. However, both the dose and the duration of supplementation are important to sustain optimum B12 status in circulation in mother-infant pairs. The observations by Duggan et al should be replicated by well-designed RCTs with optimum dose and extended beyond their 6 wk postpartum observation period. To investigate benefits of vitamin B12 supplementation, including effects on anemia, immune function and breast milk, we have conducted a pilot study in which Bangladeshi women (n=68, 18-35 y, Hb <110 g/L, 11-14 wk pregnant) were randomized to 250 µg B12/day or a placebo through 3 mo postpartum. Both groups also received 400 µg folic acid + 60 mg iron daily as standard of care. A high prevalence of deficient and marginal status of B12 was reported in early pregnancy. Furthermore, maternal deficiency predicted poorer infant B12 status through 3 mo postpartum. These observations imply that it is critically important to assess the adequacy of the vitamin in maternal and infant

plasma and breast milk in response to adequate pre- and post-natal doses of B12, in populations with a high prevalence of deficiency.

In a randomized study in Norway, intramuscular injection of 400 µg of vitamin B12 to infants (n = 54) at 6 weeks raised serum B12 (IQR: 291–497 pmol/L), and lowered MMA (from 0.58 to 0.20 µmol/L) and tHcy (from 7.46 to 4.57 µmol/L) at 4 months, compared to a group (n=51) that received no intervention [86]. Despite the high prevalence of vitamin B12 deficiency amongst pregnant and breastfeeding women and their infants, there is a paucity of information on infant development in response to vitamin B12 supplementation during pregnancy or infancy. A recent double-blind, randomized controlled trial in infants (<8 months of age) demonstrated credible evidence that intramuscular injection of 400 µg hydroxycobalamin resulted in B12 repletion, improvements in motor function and reduced regurgitations linked to neuromuscular coordination [87]. This apparent beneficial effect of vitamin B12 administration during infancy and its underlying mechanism requires more advanced investigations.

Discussion

The highlights of the review are increased B12 requirements during pregnancy and lactation, adverse pregnancy outcomes and short and long term consequences of B12 deficiency on child health. The review shows that the reference range for vitamin B12 status during pregnancy/lactation and the optimum dose to rectify the deficiency are still not clear and need more research. Very few RCTs have been conducted with inconclusive data on appropriate biomarkers of B12 status and short- and long-term health outcomes.

It is well recognized that requirement for B12 increases during pregnancy and lactation. Vitamin B12 needs during pregnancy and infancy are so high that it is virtually impossible for these to be met through diet alone, especially in low and middle income countries.

Thus, in population with high risk of B12 deficiency to reduce the gap between needs and intake, it is not imprudent to propose increased intake of B12 during pregnancy and lactation either through supplementation or via fortified food.

There have been minimal investigations into the effects of vitamin B12 supplementation during pregnancy, lactation or infancy on maternal and infants' health outcomes. Given the potential adverse effects of vitamin B12 deficiency on maternal and infant development, it is important to define B12 deficiency during each trimester using appropriate cut off. It is also important to conduct rigorously designed RCTs to find the optimum dose of B12 supplement required to replenish the deficiency, to understand factors that may explain variations in response to different doses of supplementation and impact of supplementation on metabolomic, epigenetic, immune and endocrine measures that influence the overall health outcomes in mothers and children. The long-term implication of reduced vitamin B12 status in children born to vitamin B12 deficient mothers and its underlying mechanism is not well understood and extended follow-up studies on metabolic pathways and neurodevelopment of children are required to answer these interesting questions.

Worldwide, an integrated policy has been adopted to reduce the incidence of NTD, preferably by iron-folic acid supplementation, but the high prevalence of deficiency and depletion of vitamin B12 during pregnancy and infancy leading to increase risk of neural tube defects, delayed neurological development, low birth weight and higher disease risk later in life, has received less attention by policy makers. No unified guidelines are available to assist obstetricians or pediatricians to prescribe optimum doses of vitamin B12 during pregnancy and postpartum. From a public health point of view, the identification of exact strategy to reduce vitamin B12 deficiency is of prime interest. However, defining an appropriate strategy for the prevention of deficiency critically depends on evidence based results. Most of the reported studies show high prevalence of vitamin B12 deficiency in low and middle income populations. However, there is a lack of adequately designed RCTs to evaluate the functional outcomes of B12 supplementation. Thus, in conclusion, there is an urgent need to advance our knowledge in a population specific manner to further improve the current guidelines of nutritional interventions for pregnant and lactating women that has direct relevance to child health.

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