



Effects of Vitamin B12 in Neonates and Young Infants

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Abstract

Vitamin B12 (cobalamin) is an essential coenzyme for nucleic acid synthesis. Animal protein is the major dietary source of vitamin B12. Deficiency of vitamin B12 leads to megaloblastic anemia, degeneration of the brain, spinal cord, peripheral nerves, and abnormalities of epithelial tissues. Two factors are necessary for the cure of megaloblastic anemia: one in food (extrinsic factor) and one in gastric juice (intrinsic factor). The extrinsic factor is vitamin B12. Intrinsic factor (a glycoprotein secreted by gastric parietal cells) ensures cobalamin absorption by receptors in the terminal ileum. Vitamin B12 is actively transported across the placenta. Neonates have high serum levels and significant liver stores of vitamin B12.

The neonates born to mothers with deficiency of vitamin B12 have deficiency of this vitamin. Pregnant women in resource-poor areas have low vitamin B12 status which is associated with adverse pregnancy outcomes, including anemia, low birth weight, and intrauterine growth retardation. Supplementation of vitamin B12 had significantly higher plasma of vitamin B12 in mothers and neonates. A single intramuscularly injection of vitamin B12 of between 250 µg and 1mg and a dietary intake of 1 µg/kg per day vitamin B12 is sufficient to combat vitamin B12 deficiency. Mean DNA damage scores in infants with vitamin B12 deficiency and their mothers were significantly higher before than after supplementation with vitamin B12. There were correlations between the infants' and their mothers' DNA damage scores. The aim of this study is the review of the effects of vitamin B12 in neonates and young infants.

Key Words: Deficiency, Effects, Infant, Neonate, Vitamin B12.

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1-INTRODUCTION

Vitamin B12 and folic acid are dietary essential (1). A deficiency of vitamin B12 impairs DNA synthesis in any cell in which chromosomal replication and division are taking place. An early sign of deficiency of these vitamins is megaloblastic anemia. The main storage site for vitamin B12 is the liver. Deficiencies in vitamin B12 can occur due to the lack of intrinsic factor (pernicious anemia), malabsorption of vitamin B12, deficiencies in the diet, or metabolism of vitamin B12 by intestinal parasites. The clinical presentation of vitamin B12 deficiency is typically a megaloblastic anemia. The diagnosis of vitamin B12 deficiency is confirmed by a low plasma concentration of this vitamin. Prompt replacement of vitamin B12 is indicated in patients who are found to be deficient (2).

Breastfed infants whose mothers have unrecognized pernicious anemia occasionally become B12 deficient as do a few whose mothers are on deficient vegetarian diet. Older children occasionally become deficient because of malabsorption. Pharmacological doses are beneficial in several rare (autosomal recessive) disorders of cobalamin (vitamin B12) transport and metabolism.

Vitamin B12 is a water-soluble vitamin that is actively transported across the placenta. Neonates have high serum levels and significant liver stores at birth. Meat and milk are the main dietary sources. Toxicity has not been described. Absorption requires binding to intrinsic factors (a protein secreted by the stomach) recognition of the complex by receptors in the terminal ileum and release into the portal circulation bound to transcobalamin II. Ileal absorption can be affected by surgery for necrotizing enterocolitis, while congenital transcobalamin II deficiency can also affect tissue delivery. The first sign of deficiency is neutrophil hypersegmentation. Megaloblastic anemia

develops, and severe deficiency causes neurologic damage that can be irreversible. A high folic acid intake can mask the hematological signs of vitamin B12 deficiency. Intrinsic factor failure causes pernicious anemia. The active ingredient (cyanocobalamin) was finally isolated in 1948, and a bacterial source of production developed the following year (3).

Cobalamin is released from transcobalamin II within target cells and converted to adenosylcobalamin or methylcobalamin cofactors, respectively, for methylmalonyl mutase and methionine synthase. Rare genetic defects can impair cobalamin metabolism at various stages. Patients can present at any age from 2 days to 5 years with symptoms ranging from vomiting and encephalopathy to mental delay and failure to thrive. Investigations may show a megaloblastic anemia, methylmalonic aciduria and/or homocystinuria. The investigation needs to be conducted when the patient is well and on a constant protein intake. Hydroxocobalamin 1 mg is given daily by intramuscular injection for five consecutive days and methylmalonate excretion measured before, during and after the intervention (3).

Patients with isolated homocystinuria and low or normal plasma methionine concentrations are also likely to have a cobalamin defect and should have a similar trial of vitamin B12. Patients with these conditions who are acutely unwell should be started on a 1mg dose daily intramuscularly. Treatment should be accompanied by other measures appropriate to the specific defect, such as protein restriction, carnitine and/or betaine under the guidance of a consultant experienced in the management of metabolic disease (3).

The active coenzymes methylcobalamin and 5-deoxyadenosylcobalamin are essential for cell growth and replication. Methylcobalamin is required for the

conversion of homocysteine to methionine and its derivative S-Adenosylmethionine (SAME). In addition, when concentrations of vitamin B12 are inadequate, folate becomes "trapped" as methyltetrahydrofolate to cause a functional deficiency of other required intracellular forms of folic acid. The hematologic abnormalities in vitamin B12-deficient patients result from this process. 5-Deoxyadenosylcobalamin is required for the re-arrangement of methylmalonyl CoA to succinyl CoA. Humans depend on exogenous sources of vitamin B12. In nature, the primary sources of vitamin B12 are certain microorganisms that grow in soil, sewage, water, or the intestinal lumen of animals that synthesize the vitamin B12. Vegetable products are free of vitamin B12. Despite this, strict vegetarians rarely develop vitamin B12 deficiency (4).

A neonate with elevated propionylcarnitine on the newborn screen was found to have methylmalonic acidemia due to vitamin B12 deficiency. The mother was also vitamin B12 deficient. This case illustrates the utility of expanded newborn screening for the detection of vitamin B12 deficiency, allowing prompt treatment and prevention of potential sequelae (5).

2- MATERIALS AND METHODS

2-1. Literature Search

The following databases were searched for relevant papers and reports: MEDLINE, CINAHL, Embase, Google scholar and PubMed as search engines; February 2016 was the cutoff point. Key references from extracted papers were also hand-searched.

2-2. Search Terms

Combinations of search terms from three categories ("Neonates" keyword AND "Vitamin B12" keyword AND "Effects vitamin B12 neonate" keyword AND "Infants" keyword), were used to search for the relevant literature. In addition, the

book Neonatal Formulary (3) was consulted.

3-RESULTS

3-1. Dietary deficiency

Give a single intramuscular injection of between 250 µg and 1 mg, and then ensure that the diet remains adequate (1 µg/kg a day is sufficient) (3).

3-2. Absorptive defects

Malabsorption syndrome is treated with a 1mg of hydroxocobalamin intramuscularly at monthly intervals, but 1mg three times a week is usually given in transcobalamin II deficiency during the first year of life, later reduction to 1mg once a week with hematological monitoring (3).

3-3. Metabolic disease

The initial maintenance dose is 1mg daily intramuscularly irrespective of weight, but this can often be reduced later to one to three injections a week, with biochemical monitoring to ensure that there is no deterioration. Oral hydroxocobalamin (1-20 mg per day) is sometimes substituted, but is usually less effective because the intestinal tract's absorptive capacity becomes saturated (3).

A 9-month-old exclusively breast-fed infant of a strict vegetarian mother who had excluded all animal proteins from her diet. The patient's symptoms included dystrophy, weakness, muscular atrophy, loss of tendon reflexes, psychomotor regression and hematological abnormalities. Biochemical investigations revealed severe methylmalonic aciduria in the mother and low concentrations of serum vitamin B12 in both infant and mother (6).

Pregnant women in resource-poor areas are at risk of multiple micronutrient deficiencies, and indicators of low vitamin B12 status associated with adverse pregnancy outcomes, including anemia,

low birth weight, and intrauterine growth retardation. Duggan et al. (7) evaluated whether daily oral vitamin B12 supplementation during pregnancy increases maternal and infant measures of vitamin B12 status. These authors performed a randomized, placebo-controlled clinical trial. Pregnant women <14 weeks of gestation in Bangalore, India, were randomly assigned to receive daily oral supplementation with vitamin B12 (50 µg) or placebo through 6 week postpartum. All women were administered iron and folic acid supplements throughout pregnancy. One hundred eight-three women were randomly assigned to receive vitamin B12 and 183 to receive placebo. Compared with placebo recipients, vitamin B12 supplemented women had significantly higher plasma vitamin B12 concentrations at both the second (median vitamin B12 concentrations: 216 versus 111 pmol/l; $P=0.001$) and third (median: 184 versus 105 pmol/l; $P=0.001$) trimester. At 6 weeks postpartum, median breast milk vitamin B12 concentrations was 136 pmol/l in vitamin B12 supplemented women versus 87 pmol/l in the placebo group ($P=0.000$). Among vitamin B12 supplemented women, the incidence of delivering an infant with intrauterine growth retardation was 33 of 131 (25%) versus 43 of 125 (34%) in those administered placebo ($P=0.11$). In a subset of infants tested at 6 weeks of age, median plasma vitamin B12 concentrations were 199 pmol/l in those born to supplemented women versus 139 pmol/l in the placebo group ($P=0.01$). Infant plasma methylmalonic acid and homocysteine concentrations were significantly lower in the vitamin B-12 group as well. Oral supplementation of urban Indian women with vitamin B12 throughout pregnancy and early lactation significantly increases vitamin B12 status of mothers and infants. It is important to determine whether there are correlations between these findings and neurologic and metabolic functions.

Besides the inherited form, vitamin B12 deficiency may be due to diet restrictions or abnormal absorption. The spread of newborn screening programs worldwide has pointed out that non-inherited conditions are mainly secondary to a maternal deficiency. Scolamiero et al. (8) studied seven cases of acquired vitamin B12 deficiency detected during a newborn screening project. Moreover, these authors evaluated vitamin B12 and related biochemical parameters status on delivering females to verify the consequences of newborns of eventually altered parameters. A total of 35,000 newborns were screened; those showing altered propionyl carnitine underwent second-tier tests for methylmalonic acid on dried blood spot. Subsequently, newborns positive to the presence of methylmalonic acid on dried blood spot and their respective mothers underwent further tests: serum vitamin B12, holo-transcobalamin, folate and homocysteine; newborns were also tested for urinary methylmalonic acid content. A control study was conducted on 203 females that were tested for the same parameters when admitted to hospital for delivery. Approximately 10% of the examined newborns showed altered propionyl carnitine. Among these, seven cases of acquired vitamin B12 deficiency were identified (70% of the methylmalonic acid positive cases). Moreover, the data show a high frequency of vitamin B12 deficiency in delivering females (approximately 48% of examined pregnant). These authors suggest monitoring vitamin B12 and holo-transcobalamin until delivery and reconsidering the reference interval of vitamin B12 for a better identification of case risks. Finally, newborns from mothers with low or borderline levels of vitamin B12 should undergo second-tier tests for methylmalonic acid; in the presence of methylmalonic acid they should be supplemented with vitamin B12 to prevent adverse effects related to vitamin B12

deficiency. Low maternal vitamin B12 status is a risk factor for various adverse pregnancy outcomes. Dayaldasani et al. (9) measured vitamin B12 levels in women during the first trimester of pregnancy, and evaluated predictors of these concentrations, and studied their relationship with newborn screening results. Vitamin B12 concentrations were evaluated in 204 women during the first trimester of pregnancy and possible confounding factors were analyzed. After giving birth, data of their neonates (n=189) were collected (gender, gestational age, birth weight) and the acylcarnitine profile obtained by tandem mass spectrometry during newborn screening was analyzed. To assess the effects of the variables on vitamin B12 serum concentrations and newborn screening markers, stepwise multiple linear regression models were used. The mean serum concentration of vitamin B12 was 370.8 pmol/l. Vitamin B12 concentrations were significantly lower in smokers (P=0.02), and in women with low met consumption (P=0.040).

There was a significant inverse correlation between mothers' vitamin B12 concentrations and their children's propionylcarnitine (r=0.24; P=0.001), propionylcarnitine to acetylcarnitine ratio (r= -0.23; P=0.002) and propionylcarnitine to palmitoylcarnitine ratio levels (r=0.20; P=0.006). Newborn screening markers (propionylcarnitine, propionylcarnitine to acetylcarnitine ratio, and propionylcarnitine to palmitoylcarnitine ratio) present an inverse correlation with maternal vitamin B12 status in the first trimester of pregnancy. Regarding factors that may influence maternal vitamin B12 levels during the first trimester, smoking seems to have a negative effect, and met consumption a positive effect.

Folic acid supplementation in those with a low vitamin B12 intake or status may have adverse effects. These effects are unknown with regard to birth outcome in pregnant

Indian women who are routinely supplemented with a high dose of folic acid. Dwarkanath et al. (10) examined the association of unbalanced vitamin B12 and total folate (folic acid supplement + dietary folate) intakes during pregnancy with outcomes in small-for-gestational-age infants. Low intake of dietary vitamin B12 intake (<1.2 µg per day) assessed by a food-frequency questionnaire in the first, second, and third trimester of pregnancy was 25%, 11%, and 10%, respectively. Multivariate log binomial regression showed that low vitamin B12 and folate intakes in the first trimester were independently associated with a high risk of small-for-gestational-age neonates. In a subgroup of women with high supplemental folic acid intakes in the second trimester, those with the lowest tertile of vitamin B12 to folate ratio had a higher risk of small-for-gestational-age infant outcome than did those in the highest tertile (adjusted relative risk: 2.73; 95% confidence interval: 1.17, 6.37). A similar trend was observed in the analysis of blood micronutrient status in a random subset (n=316) of the sample. These findings suggest that, in addition to vitamin B12 and folate deficiencies alone, there may be adverse birth outcomes associated with unbalanced vitamin B12 and folate intakes or status during pregnancy. These findings have important implications for the antenatal vitamin B12 supplementation policy in India.

Abraham et al. (11) studied the association between maternal vitamin B12 levels and fetal growth restriction. The nested case-control study of low-risk women attending the antenatal clinic had their blood samples taken and stored at 28 to 31 weeks of gestation. They were followed until delivery. Fifty-eight delivering neonates < 2,500 grams were taken as cases and an equal number of controls delivering neonates > 2,500 grams were taken from the same cohort. Their B12 levels were

assayed and studied for statistical significance. The baseline characteristics of both groups were similar. The number of women with serum vitamin B12 < 200 pg/ml were similar in both groups: 33% versus 29% ($P=0.84$). Type of kitchen fuel used was taken as a surrogate marker for socioeconomic status. More women in the cases used non-liquid petroleum gas kitchen fuels such as kerosene and wood than in controls, 35% versus 19% ($P=0.06$). No association between maternal vitamin B12 levels and fetal growth restriction was found in this study. Low birth weight neonates were more common in women of low socioeconomic status.

Minnet et al. (12) performed a study to assess the effects of DNA damage and vitamin B12 deficiency. The study group included 32 children (mean age= 44 ± 58 months) and 27 mothers (mean age= 30.4 ± 5.3 years). The control group contained 30 healthy children and 25 mothers. DNA strand breaks in peripheral blood mononuclear leukocytes were assayed by single-cell alkaline gel electrophoresis before and 8 days after the first injection of vitamin B12. Mean DNA damage scores in children with vitamin B12 deficiency and their mothers were significantly higher before treatment than those after treatment. The DNA damage scores of children after treatment were still significantly higher than controls. There were significant negative correlations between the children and their mothers in terms of vitamin B12 levels and DNA damage scores ($r=0.3$; $P=0.02$; $r=0.58$; $P=0.002$, respectively). There were correlations between the children's and their mothers' DNA damage and severity of vitamin B12 deficiency, suggesting that the children and their mothers may play a role in the scarcity of nutrition vitamin B12. DNA damage is increased in children with vitamin B12 deficiency and in their mothers. DNA damage scores were significantly improved through vitamin

B12 therapy 8 days after the first injections however, they were still significantly higher than those of controls.

Premature infants, especially those with birth weight < 1,500 grams often suffer from anemia of prematurity. Haiden et al. (13) hypothesized that combined administration of vitamin B12 and folate with erythropoietin and iron would enhance erythropoietin-induced erythropoiesis. In a randomized, controlled trial, 64 premature infants (birth weight: 801 to 1,300 grams) receiving erythropoietin and iron supplementation were assigned randomly to receive either vitamin B12 (3 $\mu\text{g}/\text{kg}$ per day), and folate (100 $\mu\text{g}/\text{kg}$ per day) (treatment group) or a lower dose of folate (60 $\mu\text{g}/\text{kg}$ per day) (control group). During the 4-week observation period, vitamin B12 and folate enhanced erythropoietin-induced erythropoiesis significantly, as indicated by a 10% increase in red blood cell counts, compared with folate alone. Hemoglobin and hematocrit levels remained stable in the treatment group, whereas they decreased in the control group. Vitamin B12 levels in the treatment group increased over baseline and control values, whereas red blood cell folate levels were comparable between the groups. Subsequent analysis showed slight non-significant differences in baseline red blood cell count, hemoglobin level, hematocrit level, and mean corpuscular volume values, which must be addressed as a limitation. With the limitation of a slight imbalance in baseline data between the study groups, combined therapy with vitamin B12, folate, erythropoietin, and orally and intravenously administered iron seemed more effective in stimulating erythropoiesis among premature infants, compared with erythropoietin, iron, and low-dose folate alone.

Minet et al. (14) aimed at providing a reference range for total serum homocysteine in neonates and to explore

the relation of total serum homocysteine to serum vitamin B12, and the product of the transsulfuration pathway, and nutritional factors. Total serum homocysteine, folate, vitamin B12, and vitamin B6 were measured in 123 healthy, breast-fed neonates. The influence of nutrition (formula or human milk) on these variables was investigated in 60 infants. The mean \pm SD total serum homocysteine concentration was 7.8 \pm 3.1 μ mol/l. Total serum homocysteine showed a negative linear correlation with log vitamin B12 ($r = -0.64$; $P = 0.001$), red blood cell folate ($r = -0.33$; $P = 0.001$), and a correlate positively with cysteine ($r = 0.36$; $P = 0.001$). The strongest linear correlation was found between total serum cysteine and the ratio of log cysteine to log vitamin B12 ($r = 0.71$ $P = 0.000$). These authors found more neonates with probable tissue deficiencies of vitamin B12 and folate on the basis of total serum homocysteine measurements than was expected from the analysis of serum vitamin B12 concentrations alone (15.4% compared with 9.7%). Breast-fed infants had significantly lower vitamin B12 concentrations and significantly higher serum homocysteine and cysteine concentrations and ratios of log cysteine to log vitamin B12 than did formula-fed infants ($P = 0.001$). Total serum cysteine can be used as a functional indicator of vitamin B12 and folate status in neonates. The ratio of cysteine to vitamin B12 can be used as an additional index of impaired intracellular homocysteine metabolism. Total serum homocysteine concentration in infants, are affected by nutritional factors.

4-DISCUSSION

Vitamin B12 is required for efficient nucleic acid biosynthesis, acting as a coenzyme for several reactions involving the metabolism of single-carbon groups and folate. It is also necessary for nervous system functions. The main source of vitamin B12 is animal protein. A deficiency state from an inadequate supply

of this vitamin is found in only the strictest of vegetarians. The main storage site for vitamin B12 is the liver. Deficiencies in vitamin B12 can occur due to the lack of intrinsic factor (pernicious anemia), malabsorption of vitamin B12, deficiencies in the diet, or metabolism of vitamin B12 by intestinal parasites. The clinical presentation of vitamin B12 deficiency is typically a megaloblastic anemia (2).

Pregnant women in resource-poor areas are at risk of low vitamin B12 status and their neonates have anemia, low birth weight and intrauterine growth retardation. The therapy of neonates with vitamin B12 deficiency is a single injection of between 250 μ g and 1 mg, and then a diet containing 1 μ g/kg of vitamin B12. The patient's symptoms of vitamin B12 include dystrophy, weakness, muscular atrophy, loss of tendon reflexions and hematological abnormalities.

Duggan et al. (7) observed that a supplementation of 50 μ g of vitamin B12 to pregnant women 14 weeks of gestation significantly increased plasma B12 concentrations at both the second and third trimester of pregnancy compared with pregnant women receiving placebo. In infants tested at 6 weeks of age median plasma vitamin B12 was significantly higher in infants born to supplemented women than in infants whose mothers received placebo. At 6 weeks postpartum, breast milk concentrations of vitamin B12 were significantly higher than the milk of women receiving placebo.

Low maternal vitamin B12 status is a risk factor for various adverse pregnancy outcomes. There was a significant inverse correlation between mothers' vitamin B12 concentrations and their children's propionylcarnitine, propionylcarnitine to acetylarnitine ratio and propionylcarnitine to palmitoylcarnitine ratio levels. Newborn screening markers (propionylcarnitine, propionylcarnitine to acetylarnitine ratio, and propionylcarnitine to

palmitoylcarnitine ratio) present an inverse correlation with maternal vitamin B12 status in the first trimester of pregnancy (9). Multivariate log binomial regression showed that low vitamin B12 and folate intakes in the first trimester of pregnancy were independently associated with high risk of small-for-gestational-age neonates. The women with high supplemental folic acid intake in the second trimester and with the lowest tertile of vitamin B12 to folate ratio had a higher risk of small-for-gestational-age infant outcome than did those with the highest tertile of vitamin B12 (10).

Minnet et al. (12) assessed the effects of DNA damage and vitamin B12 deficiency. Mean DNA damage scores in infants with low vitamin B12 deficiency and their mothers were significantly higher before treatment than those after treatment. There were significant negative correlations between the infants and their mothers in terms of vitamin B12 levels and DNA damage. There were correlations between the infants' and their mothers' DNA damage and severity of vitamin B12 deficiency. DNA damage is increased in infants with vitamin B12 deficiency and in their mothers. Premature infants, especially those with birth weight < 1,500 grams often suffer from anemia of prematurity. In a randomized, controlled trial, premature infants received erythropoietin and iron and were assigned randomly to receive vitamin B12 (3 µg/kg per day) and folate (100 µg/kg per day) (treatment group) or a lower dose of folate (60 µg/kg per day) (control group). During the 4-week observation period, vitamin B12 and folate enhanced erythropoietin-induced erythropoiesis significantly, as indicated by a 10% increase in red blood cell counts, compared with folate alone. Vitamin B12 levels in the treatment group increased over baseline whereas red blood cell (RBC) folate was comparable between groups (13).

5-CONCLUSION

In conclusion, vitamin B12 (cobalamin) is an essential coenzyme for nucleic acid synthesis. Animal protein is the dietary source of vitamin B12. Vegetable products are free of vitamin B12 and strict vegetarians, develops vitamin B12 deficiency. Vitamin B12 is actively transported across the placenta and neonates have high serum and liver stores of vitamin B12 at birth. The deficiency of vitamin B12 causes megaloblastic anemia, and severe deficiency causes neurologic damage that can be irreversible. To combat vitamin B12 deficiency it is sufficient to receive a single intravenous injection of between 250 and 1 mg vitamin B12 and a diet containing 1 µg/kg per day of this vitamin. Low maternal vitamin B12 status is a risk factor for various adverse pregnancy outcomes. Pregnant women with the lowest tertile of vitamin B12 to folate ratio have a higher risk of small-for-gestational-age infants. Supplementation of vitamin B12 during pregnancy increases maternal, neonatal status and breast milk concentration of this vitamin. Mean DNA damage scores in infants with vitamin B12 deficiency and their mothers were significantly higher before than after treatment with vitamin B12. There were significant negative correlations between infants and their mothers in terms of vitamin B12 and DNA damage scores. Thus vitamin B12 protects DNA damage. Although there are several studies on vitamin B12 in neonates and young infants more studies are required for an evidence-based treatment of neonates or young infants with this vitamin.

6-CONFLICT OF INTERESTS

Prof. Gian Maria Pacifici declares no conflicts of financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employments, gifts and honoraria.

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