



Prevalence of Vitamin B12 Deficiency among Pregnant Women in Samsun Province of Turkey

Samsun ve Çevresindeki Gebelerde B12 Vitamini Eksikliğinin Sıklığı

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ABSTRACT

Purpose: Vitamin B12 deficiency in infancy can cause severe demyelinating central nervous system disorder. Most cases are exclusively breast-fed infants born to mothers with deficient vitamin B12 stores. As maternal vitamin B12 stores are the main determinant of neonatal vitamin B12 status, we aimed to determine the vitamin B12 status of pregnant women at term in our region.

Material and Methods: Serum vitamin B12, folate and plasma homocysteine concentrations in addition to routine testings were measured 1-24 hour(s) before delivery in 62 pregnant women. Besides, infants born to mothers with laboratory evidence of vitamin B12 deficiency were referred to pediatric hematology unit for evaluation.

Results: Thirty-six (58.1%) women had a low serum vitamin B12 level (<200 pg/ml). The mean plasma homocysteine was found as $6.82 \pm 2.38 \mu\text{mol/L}$. Among the 36 neonates whose mothers had a low serum vitamin B12 before delivery, 20 were admitted for examination. Plasma hcy level was not available in four infants. In the remaining 16, 13 (81.3%) had both a decreased vitamin B12 level (<259 pg/ml), and an elevated homocysteine concentration (>9.99 $\mu\text{mol/L}$). All of the 20 infants examined had normal neurodevelopmental findings. Vitamin B12 supplementation resulted in normalization of vitamin B12 and homocysteine levels in all the cases.

Conclusion: The frequency of neonatal vitamin B12 deficiency secondary to maternal deficiency may be higher than thought in Samsun province of Turkey. Large-scale screening studies may reveal its prevalence more accurately, and may help in taking preventive measurements more effectively.

Key Words: neonatal, vitamin B12, homocysteine, pregnancy, maternal

ÖZET

Amaç: B12 vitamini eksikliği, süt çocukluğu döneminde ağır demiyelinizan santral sinir sistemi bozukluğuna neden olabilir. Olguların çoğu, B12 vitamini depoları yetersiz annelerden doğan ve sadece anne sütü ile beslenen bebeklerdir. Yenidoğan bebeklerde, B12 vitamini durumunun temel belirleyicisi, annenin B12 depolarıdır. Bu nedenle, çalışmamızda, bölgemizdeki term gebelerde B12 vitamini durumunu değerlendirmeyi amaçladık.

Materyal ve Metod: Toplam 62 gebe kadında, doğumdan 1-24 sa önce rutin tetkiklere ek olarak serum B12, folat ve plazma homosistein değerleri ölçüldü. Ayrıca, B12 eksikliği bulgusu olan annelerin yenidoğan bebekleri değerlendirilmek üzere çocuk hematolojisi polikliniğine yönlendirildi.

Bulgular: 36 (%58,1) gebe kadının serum B12 değeri düşük (<200 pg/ml) bulundu. Plazma homosistein (ortalama \pm SD) $6,82 \pm 2,38 \mu\text{mol/L}$ olarak hesaplandı. Annesinin serum B12 değeri doğum öncesinde düşük bulunan 36 yenidoğandan 20'si değerlendirildi. 4 bebekte plazma homosistein ölçülemedi. Kalan 16 yenidoğan bebeğin 13'ünde, düşük serum B12 (<259 pg/ml) ve yüksek plazma homosistein (>9,99 $\mu\text{mol/L}$) birlikteliği mevcuttu. Değerlendirilen 20

yenidoğanın tamamının fizik muayene bulguları normaldi. B12 replasmanı sonrasında tüm olgularda serum B12 ve plazma homosistein değerleri normal olarak bulundu.

Sonuç: Samsun ve çevresindeki yenidoğanlarda, annedeki eksikliğe ikincil B12 vitamini eksikliği, düşünüldüğünden daha sık olabilir. Geniş ölçekli tarama çalışmaları ile bu durumun prevalansı daha hassas şekilde belirlenebilir ve koruyucu önlemlerin daha etkili şekilde alınması sağlanabilir.

Anahtar Kelimeler: Yenidoğan, B12 vitamini, homosistein, gebelik, maternal

INTRODUCTION

Vitamin B12 (cobalamin) plays an essential role in human metabolism, acting as a cofactor for two important metabolic reactions: it converts methylmalonyl-CoA to succinyl-CoA (a compound metabolized by the Krebs cycle to produce energy) and homocysteine (hcy) to the essential amino acid methionine¹. Deficiency of vitamin B12 leads to the accumulation of methylmalonic acid (MMA) and hcy, and the onset of clinical hematological and neurological manifestations, mainly reversible bone marrow failure and demyelinating central nervous system disease^{1,2}. Vitamin B12 is synthesized by microorganisms, and is present only in foods of animal origin, such as meat, egg, fish and milk.

Vitamin B12 is required for the development and myelination of the central nervous system in the early years of life besides for the maintenance of its normal function². Breast-feeding in infants born to vitamin B12-deficient mothers is the main cause of its deficiency during infancy which may manifest as failure of brain development in addition to overall growth and development, usually between 4 and 6 months of age. Imaging studies may reveal brain atrophy and delayed myelination. Anemia may be present. Replacement with vitamin B12 usually reverts the related neurological symptoms, and many infants recover fully. However, in cases with severe and prolonged duration of deficiency, irreversible neurodevelopmental deficits may occur despite therapy highlighting the importance of early diagnosis and treatment.

Pregnant women with low vitamin B12 stores can give birth to vitamin B12-deficient neonates³, and maternal vitamin B12 stores are thought to be

the main determinant of neonatal vitamin B12 status. Studies assessing vitamin B12 status in pregnant women from different regions of Turkey all found a high frequency of its deficiency^{3,4,5,6,7}. To our knowledge, no study has evaluated vitamin B12 status in expectant mothers in the Samsun province before.

MATERIALS and METHODS

The study was approved by the Institutional Ethic Committee. Sixty-two pregnant women who admitted to the Emergency Clinic of Samsun Maternity and Child Health Hospital for labor and gave their informed consent were included in this study. All of them gave birth to singleton-term babies via uneventful delivery. None had a chronic disease such as diabetes or hypertension. Age, educational status, detailed nutritional habits including the frequency of consumption of animal products, weight gain and routine vitamin supplementation during pregnancy; socioeconomic status of the family; birth weight, length and head circumference of the newborn babies were all recorded. Blood was taken 1-24 h before delivery, and serum vitamin B12, folate and plasma hcy levels were measured in addition to routine testings for complete blood count and serum ferritin.

For serum vitamin B12 and folate measurement, blood in the sterile tubes without any anticoagulants collected for ferritin measurement was used, whereas for hcy measurement, blood samples in EDTA-tubes were transferred on ice to the laboratory and centrifuged immediately. Vitamin B12, folate and hcy testings were all performed using the chemiluminescent technique by 2000 (Immulate Diagnostic Products Corporation, 2000).

We accepted a hemoglobin level lower than 110 g/L as anemia in pregnant women. Our laboratory uses 200 pg/ml, 3.0 ng/ml and 12.0 µmol/L as cut-off points for serum vitamin B12, folate and plasma hcy, respectively. Newborn infants born to mothers with a low serum B12 or high plasma hcy were referred by the obstetrician to the Pediatric Hematology and Oncology Unit of our hospital. Serum vitamin B12, folate and plasma hcy of the admitting newborns were measured, and neonatal reference ranges found recently by Bailey et al.⁸ were used for these variables.

RESULTS

Sixty-two pregnant women with a mean age of 27.2 ± 5.5 years who completed their 37th gestational week were included in this study. The mean hemoglobin was found 120.8 ± 11.3 g/L (range: 96.0-145.0 g/L). Twelve (19.4%) of the mothers had mild anemia, all being normocytic, whereas 5 mothers (8.1%) had macrocytosis (MCV >93 fL) with hemoglobin values in the normal range. The mean serum ferritin was 16.9 ± 15.7 mg/dl (3.45-71.4).

Thirty-six (58.1%) of the mothers had a serum vitamin B12 level lower than 200 mg/dl, whereas 15 (24.2%) had a level lower than 150 mg/dl. None but one had a low serum folate level. The mean plasma hcy was 6.82 ± 2.38 (range: 2.88-13.80) µmol/L. Only two mothers had a plasma hcy concentration above the upper limit of our laboratory. Among the thirty-six neonates whose mothers had a serum vitamin B12 lower than 200 mg/dl and/or a plasma hcy higher than 12.0 µmol/L, twenty (16 male, 4 female; age: 5-17 days) were seen in the Pediatric Hematology and Oncology Unit, all of them being exclusively breast-fed. Serum vitamin B12 and folate were measured in all, whereas plasma hcy level was not available in 4 infants. In Table 1, serum vitamin B12, folate and plasma hcy concentrations of the mothers and the 20 available newborn babies are presented. Among the 16 neonates with vitamin B12 and hcy levels, 13 (81.3%) had both a low vitamin B12 and

an elevated plasma hcy level. Table 2 summarizes the number of the infants with normal and abnormal vitamin B12 and hcy levels.

In the dietary history, the number of the mothers reporting to have consumed specific type of animal-source food once every month or less often was as follows: red meat: 30 (48.4%); chicken: 14 (22.6%); eggs: 8 (12.9%); fish: 26 (41.9%); milk: 16 (25.8%); cheese: 3 (4.8%); yoghurt: 1 (1.6%). Besides, 16 (25.8%) of the mothers reported not having used the folic acid and/or multivitamin supplements regularly during their pregnancy prescribed routinely by their obstetrician.

In the physical examination, none of the 20 neonates had any abnormal neurodevelopmental finding. All of the mothers and their infants with laboratory findings of vitamin B12 deficiency received vitamin B12 supplementation. In their last visit, both serum vitamin B12 and plasma hcy levels were found to be in the normal range in all of the cases.

DISCUSSION

The results of this study indicate that subclinical neonatal vitamin B12 deficiency secondary to low maternal vitamin B12 stores may be more frequent than previously thought in Samsun province of Turkey. Being essential for the developing central nervous system, even moderate B12 deficiency has been found to be harmful for infants⁹. In vitamin B12-deficient cases, neurological signs may precede hematological findings associated with megaloblastic anemia¹⁰. Through prevention and early treatment of vitamin B12 deficiency in infancy, irreversible neurological damage can be prevented.

Although our laboratory uses 200 pg/ml as the lower threshold for serum vitamin B12 in general, vitamin B12 level is known to drop during pregnancy. Factors such as hemodilution, hormonal changes, altered renal function, changes in the cobalamins attached to holotranscobalamin and fetomaternal transfer are thought to contribute

to this physiological decline^{11,12,13,14}. In this study, 36 (58.1%) of the expectant mothers had a serum vitamin B12 level lower than 200 pg/ml, and their infants were referred to our unit by the obstetrician. However, in spite of being the most frequently used test in confirming the diagnosis of vitamin B12 deficiency and an extremely low level (<100 pg/ml) usually reflects true deficiency, both false negative and false positive values can occur with serum vitamin B12 assays, occurring in up to 50% of tests^{10,15,16}. This may be explained by the fact that only 20% of the total measured vitamin B12 is on transcobalamin which is the cellular delivery protein¹⁷. Measurement of MMA or hcy is useful in making the diagnosis of vitamin B12 deficiency². The levels of both of these metabolites are markedly elevated in the vast majority (98%) of patients with clinical deficiency, including those with only neurological manifestations in the absence of hematological findings. Owing to the limitations of vitamin B12 assays in confirming the diagnosis of vitamin B12 deficiency, measurement of one or both of these two metabolites is recommended in subjects with suspected vitamin B12 deficiency. Besides, increased levels of plasma MMA and hcy are usually detectable in individuals with vitamin B12 deficiency before any hematological or neurological signs become evident¹⁸. For this reason, we measured plasma hcy in addition to serum vitamin B12 in expectant mothers and infants born to deficient women.

The mean plasma hcy level of the expectant mothers in this study was 6.82 $\mu\text{mol/L}$, only two women having values above the upper limit of our laboratory. However, hcy levels have been shown to be lower in pregnancy than in the non-pregnant state previously^{19,20,21}. Hormonal changes, increased protein anabolism in pregnancy, uptake of hcy by the fetus, hemodilution, changes in renal hemodynamics, decrease in albumin which binds 70% of the hcy in human plasma and folic acid supplementation may be responsible for this change. Besides, the decrease in hcy during pregnancy has been thought to be a possible

compensatory mechanism to prevent undesired thrombosis^{20,22}. In a study from Canada including 468 mothers from whom blood was collected within 48 hours after delivery, mean plasma hcy was found as 5.59 $\mu\text{mol/L}$ ²³. In another study from USA, similar results were obtained (5.43 \pm 1.40 $\mu\text{mol/L}$)²⁴. The lower hcy levels found in those two studies may be explained by food fortification and the effect of nutritional and lifestyle factors on hcy concentrations. We think that nutritional factors secondary to low socioeconomic status and the habit of consuming green vegetables predominantly instead of animal products in our region may explain the higher hcy levels of our subjects.

In their study including 68 pregnant women from Istanbul, Turkey, Haliloglu et al. found serum hcy level to be lowest in the third trimester of pregnancy (4.15 \pm 1.23 $\mu\text{mol/L}$), becoming higher after delivery²⁰. Expectant mothers in our study had a higher mean hcy value than those pregnant women. Our hospital is a state hospital, and many of the mothers have low socioeconomic status and educational level. Indeed, the dietary history was quite poor in most of the mothers included, almost half of them reporting have consumed red meat once every month or less often.

Pregnant women with low vitamin B12 stores can give birth to vitamin B12-deficient neonates³, and maternal vitamin B12 stores are thought to be the main determinant of neonatal vitamin B12 status. In addition, if the mother does not consume vitamin B12-containing products adequately after birth or has a disorder causing vitamin B12 deficiency like pernicious anemia, vitamin B12 content of her breast milk will be low, which will further aggravate the deficiency in the infant if exclusively breast-fed.

In their study, Monsen et al. found elevated plasma hcy in neonates with low vitamin B12 levels at 96-108 hours after birth²⁵. Karademir et al. found that plasma hcy measurement at birth and in infants younger than 6 months of age to be useful for identifying vitamin B12 deficiency²⁶. On the

contrary, urinary MMA was not found useful in the diagnosis of vitamin B12 deficiency in that study. It exhibited a wider range in neonates explained mainly by the immaturity of enzyme systems in tissues related to MMA metabolism. In our study, we used plasma hcy in addition to serum vitamin B12 for determining vitamin B12 status of the neonates.

In this study, 13 (81.3%) of the 16 infants were found as vitamin B12-deficient based on the combination of low vitamin B12 and high plasma hcy levels, whereas 1 had only an elevated hcy, and 2 had only a low vitamin B12 level (Table 2). Increased hcy may be secondary to other causes than vitamin B12 deficiency such as folate deficiency or homocystinuria, however, in our case with abnormal hcy alone, serum folate was normal and hcy level normalized after vitamin B12 supplementation indicating deficiency of vitamin B12 to be the underlying cause. Alternatively, low sensitivity of serum vitamin B12 measurement for the diagnosis of vitamin B12 deficiency may be the explanation. On the contrary, a low serum vitamin B12 level in the absence of an elevated hcy may indicate false positivity.

In this study, the remaining 16 among the 36 newborn infants whose mothers had a low serum vitamin B12 before delivery were not admitted to us, and a serum vitamin B12 level ≥ 200 pg/ml in the expectant mothers actually does not rule out vitamin B12 deficiency in the mother and her fetus (infant). We think that more reliable reference ranges for the variables related to vitamin B12 during pregnancy should be established to identify and treat vitamin B12-deficient cases.

In the present study, plasma hcy levels of the expectant mothers showed a lower dispersion than the results of 16 neonates with hcy values available, from whom 14 had an increased plasma hcy concentration. Hübner et al. have reported their observation of time-dependant increase in plasma hcy in EDTA-containing whole blood after 24 h at room temperature²⁷. This is thought to occur due to the leakage of hcy (generated from S-

adenosyl-homocysteine) into plasma from erythrocytes. We asked whether the high frequency of elevated hcy finding in the neonates in our study could be due to inappropriate laboratory conditions, however, this seems to be unlikely, as the blood samples of all the mothers and neonates were handled in the same manner before laboratory measurement (transferred on ice to the laboratory and centrifuged immediately).

Campbell et al. from USA reported two asymptomatic newborns with vitamin B12 deficiency secondary to maternal deficiency who were detected incidentally¹⁸. Both infants were treated with intramuscular vitamin B12. The authors suggested adding the measurements of MMA, propionylcarnitine and hcy levels in blood spots in expanded newborn screening to detect asymptomatic newborns more reliably. Recently, the Mayo Clinic Laboratories implemented a procedure (tandem mass spectrometry) for measuring both hcy and MMA in dried blood samples in neonates²⁸. With the routine use of such a neonatal screening test which would not necessitate venipuncture, nearly all vitamin B12-deficient neonates could be identified.

All of the neonates with laboratory findings of vitamin B12 deficiency in our study had normal physical examination findings. Owing to the difficulty of diagnosing vitamin B12 deficiency in infancy due to the absence of specific findings, the potentially devastating and irreversible neurological sequela in untreated infants and a readily available treatment, routine neonatal screening for vitamin B12 deficiency in our region, perhaps in whole Turkey, seems to be justified.

The present data are a source of concern because a high proportion of newborn babies examined were found to have laboratory results consistent with subclinical vitamin B12 deficiency indicating inadequate fetomaternal transfer of vitamin B12. The prevalence of neonatal (and infantile) vitamin B12 deficiency in our region may be higher than previously thought. We think that large-scale studies evaluating the prevalence of

vitamin B12 deficiency in pregnant women and the newborn babies in our region are necessary to determine the frequency of this condition more accurately. This may help in taking preventive measures for this condition more effectively.

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Table 1. Serum vitamin B12, folate and plasma homocysteine levels of the mothers and neonates examined.

	VitB12 (pg/ml)	Folate (ng/ml)	Hcy (µmol/L) Mean ± SD (range)
<u>Mothers (n=62)</u>	<u>150 150-199 ≥200</u>	<u><3.0 ≥3.0</u>	6.82 ± 2.38 (3.11-13.80)
Number of subjects (%)	15 (24.2) 21 (33.9) 26 (41.9)	1 (1.6) 61 (98.4)	
<u>Newborns (n=20)</u>	<u><150 150-258 ≥259</u>	<u><10.6 ≥10.6</u>	19.41 ± 9.51 (6.43-46.30)*
Number of subjects (%)	1 (5.0) 15 (75.0) 4 (20.0)	3 (15.0) 17 (85.0)	

Note. * Plasma hcy was available in 16 neonates.

Table 2. Number of the sixteen infants regarding their serum vitamin B12 and plasma homocysteine levels

	Hcy normal (≤9.99 µmol/L)	Hcy elevated(>9.99 µmol/L)
Vitamin B12 normal (≥259 pg/ml)	n=0	n=1* (6.3%)
Vitamin B12 low (<259 pg/ml)	n=2† (12.5%)	n=13 (81.3%)

Notes. In 4 infants, only serum vitamin B12 level was available; 2 had low levels, whereas the other 2 had levels in the normal range.

* Serum folate was normal in that case.

† In one of these infants, plasma hcy was only slightly lower than the upper limit of the normal range (9.63 µmol/L), whereas it was 6.43 µmol/L in the other.

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