ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Investigation of the Relationship Between Maternal & Neonatal Vitamin B12 Deficiency and Neonatal Hyperbilirubinemia: A Prospective Controlled Study

Neonatal Hiperbilirübinemi ve Maternal & Neonatal Vitamin B12 Eksikliği Arasındaki İlişkinin Araştırılması: Prospektif Kontrollü Çalışma

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Abstract

Introduction: The aim in this study was to investigate the role of vitamin B12 deficiency in neonatal hyperbilirubinemia.

Materials and Methods: Term newborns who were breastfed and with hyperbilirubinemia were included in this prospective study. Those with hyperbilirubinemia were assigned to a patient group, and those without hyperbilirubinemia were assigned to a control group. The vitamin B12 levels of all newborns and their mothers were checked.

Results: A total of 154 newborns were included in the study. Vitamin B12 deficiency was significantly higher in the patient group in comparison to the control group. Similarly, vitamin B12 levels of mothers were significantly lower in the patient group. The mean bilirubin level and phototherapy need were found to be significantly higher in patients with vitamin B12 deficiency.

Conclusion: This study showed that vitamin B12 deficiency in the mother is related to vitamin B12 deficiency in the newborn, which significantly leads to neonatal hyperbilirubinemia.

Keywords
Breast milk, hyperbilirubinemia, newborn, vitamin B12 deficiency

Öz

Giriş: Bu çalışmada neonatal hiperbilirubinemi olgularında vitamin B12 eksikliğinin rolünün araşatırılması amaçlandı.

Gereç ve Yöntem: Bu prospektif çalışmaya yenidoğan sarılığı olan ve anne sütü ile beslenen term bebekler dahil edildi. Sarılığı olan olgular hasta, sarılığı olmayan olgular kontrol grubu olarak tanımlandı. Tüm bebekler ve annelerinde vitamin B12 düzeyi bakıldı.

Bulgular: Toplam 154 bebek çalışmaya dahil edildi. Vitamin B12 eksikliği hasta grubunda, kontrol grubuna kıyasla anlamlı olarak yüksek bulundu. Benzer şekilde hasta grubunun annelerinde de vitamin B12 düzeyi anlamlı düşük idi. Hasta grubunda ortalama bilirubin düzeyi ve fototerapi ihitiyacı vitamin B12 eksikliği saptanan bebeklerde anlamlı olarak yüksek olduğu görüldü.

Sonuç: Bu çalışmada annelerdeki vitamin B12 eksikliğinin yenidoğan bebeklerde vitamin B12 eksikliği ile ilişkili olduğu ve bebeklerdeki bu eksikliğin yenidoğan sarılığı gelişimine yol açtığı gösterildi.

Anahtar kelimeler
Anne sütü, sarılık, yenidoğan, vitamin B12 eksikliği

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Introduction

Neonatal hyperbilirubinemia (NH) is one of the most common clinical findings in newborns (1). It is found in about 60% of term newborns in the first week of life (2). Although it is usually temporary, NH is one of the most common causes of hospitalization in newborns.

NH etiology especially includes blood type incompatibility, enzyme deficiencies, erythrocyte membrane defects, metabolic diseases and breast milk jaundice, as well as a variety of risk factors such as race, ethnicity, sex and low birth weight, but in most cases, the underlying cause cannot be identified (1).

Vitamin B12 dissolves in water, is synthesized by microorganisms and has a symmetrical and complex structure. Among all vitamins, it is the largest and has the most complex structure. Vitamin B12 is essential for DNA synthesis and for cellular energy production. The system that is most sensitive to B12 deficiency is the hematopoietic system, especially erythropoietic series, where the cell proliferation rate is high. Red blood cell destruction causes excessive amounts of heme production, resulting in hyperbilirubinemia (3-5).

Maternal vitamin B12 deficiency has been shown to cause vitamin B12 deficiency in newborn babies (6-8). There are, however, few studies exploring the association between vitamin B12 deficiency and neonatal jaundice development.

The aim in this study was to check vitamin B12 levels in NH patients and their mothers and examine the role of vitamin B12 deficiency in NH and its impact on the severity of the disease.

Materials and Methods

Term newborns admitted to the hospital between 1 February 2018 and 31 May 2019 were included in this prospective study. The study protocol was approved by the Uludağ University Ethics Committee on the decision date of 11 January 2018 date and with the decision number of 2018-1/13. Informed parental consent was obtained for all babies.

Study Population

This study included 211 full term neonates divided into 2 groups;

Patient group: Patients with significant hyperbilirubinemia in infants ≥35 weeks gestational age (GA) is defined as a TB >95th percentile on the hour-specific Bhutani nomogram.

Control group: The control group who did not have hyperbilirubinemia consisted of sex- and age-matched healthy subjects from our clinic. Health status was determined through the subjects' medical history and parental report.

Inclusion criteria

- Gestational age 37-42 week
- Postnatal age 3-7 days
- Birth weight from 2500 gm to 3700 gm
- Good general condition
- Breast milk feeding
- Normal platelet count and WBCs and no other signs of infection

Exclusion criteria

- Blood type incompatibility
- Glucose-6-phosphate dehydrogenase (G6PDH) deficiency
- Pyruvate kinase (PK) deficiency
- Hypothyroidism
- Direct hyperbilirubinemia
- Dehydration

Clinical Features

Neonatal and maternal demographic characteristics, ages at the time of admission, phototherapy or blood exchange needs and hospitalization stays were recorded for all cases. Birth weight and body weight at admission recorded.

Laboratory Tests

Complete blood count (CBC), direct coombs, peripheral blood smears, reticulocyte and serum total bilirubin levels of all cases, as well as maternal and infant vitamin B12 levels, were checked.

Serum total and direct bilirubin levels were measured by using the spectrophotometric measurement method on an Abbott Architect C16000 device. Vitamin B12 levels were checked by using the chemiluminescent microparticle immunoassay (CMIA) method on an Abbott Architect I2000 device.

Reference Values

The total bilirubin levels of the cases were classified according to the bilirubin nomograms based on values

defined by the American Academy of Pediatrics. Those with a vitamin B12 level below 250 ng/L were considered to have vitamin B12 deficiency (9).

Treatment Protocol and Follow-up

The total bilirubin levels of all patients in the patient group were followed up. The newborns and their mothers who were diagnosed to have vitamin B12 deficiency were treated for vitamin B12.

The dose was administered as 1 drop (330 microgram) for newborns and 1 puff (1000 microgram) sublingually for their mothers every day for the first two weeks and then 3 days a week. Total bilirubin levels were checked on day 7 of the treatment, and vitamin B12 levels were checked after 1 month of treatment. Treatment was planned to be maintained.

Statistical Analysis

The SPSS SPSS program (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp.), program was used for data analysis. The descriptive statistics are presented in frequencies and percentages. The relationships between categorical variables were examined by running chi-squared tests and Fisher's Exact tests. The results were evaluated in a 95% confidence interval, and $p<0.05$ was considered significant.

Results

A total of 154 term newborns, 104 in the patient group and 50 in the control group, were included in the study.

No significant difference was found between the patient and control groups when the groups were compared in terms of their demographic characteristics (Table 1).

Vitamin B12 deficiency was found in 63.5% (n=66) of the patient group and 32% (n=16) of the control group, and the difference was significant (p=0.0001). Similarly, the mean vitamin B12 level of the patient group was also significantly lower than the control group (p=0.002). Vitamin B12 levels of the mothers in the patient group were also found to be significantly lower compared to the control group (p=0.01). Similarly, the vitamin B12 levels of the

Table 1. Demographic characteristics and laboratory tests

Demographic characteristics	Patient group n=104	Control group n=50	p
Maternal age (years), mean ± SD	28.7±5.2	28.2±5.7	0.6
Male, n (%)	47 (45.2)	30 (60)	0.6
Gestational week, mean ± SD	38.2±0.9	38.5±1.03	0.2
Birth weight (g), mean ± SD	3228±448	3316±503	0.2
Cesarean, n (%)	63 (60.6)	33 (66)	0.3
APGAR 1, median (min-max)	9 (7-10)	9 (8-10)	0.1
APGAR 5, median (min-max)	10 (8-10)	10 (9-10)	0.1
Postnatal age, mean ± SD	5.0±1.9	4.7±2.2	0.4
Laboratory tests			
Total bilirubin (mg/dL), mean ± SD	14±2.7	10.1±3.2	**0.0001**
Total bilirubin above 2 SD, n (%)	64 (61.5)	0 (0)	-
Hemoglobin (g/dL), infant, mean ± SD	15.9±2.4	16.3±1.8	0.2
Reticulocyte count (%), mean ± SD	1.5±1	0.9±0.34	**0.02**
Hemoglobin (g/dL), mother, mean ± SD	12.2±1.6	10±0	0.153
Vitamin B12 deficiency, infant n (%)	66 (63.5)	16 (32)	**0.0001**
Vitamin B12 level (ng/L), infant, mean ± SD	259±154	346±169	**0.002**
Vitamin B12 deficiency, mother n (%)	50 (48)	14 (21.9)	**0.01**
Vitamin B12 level (ng/L), mother, mean ± SD	280±136	322±138	0.07

SD: Standard deviation

mothers were found to be low in the patient group in comparison to the control group. While there was no significant difference between the groups in terms of the hemoglobin levels, the reticulocyte counts were significantly higher in the patient group (p=0.02) (Table 1).

A total of 64 mothers had B12 deficiency. B12 deficiency was found in the babies of 54 of these mothers and 50 of these babies were in the patient group (Figure 1).

When the patient group was categorized based on low and normal vitamin B12 levels, it was found that there was no significant difference in terms of the demographic characteristics (Table 2).

The mean bilirubin levels, severe hyperbilirubinemia (>15 mg/dL) and need for phototherapy were significantly higher in with vitamin B12 deficiency patient. No patient needed exchange transfusion. In the patients with vitamin B12 deficiency, the need for for >48 hours of phototherapy was higher (Table 2).

An ROC analysis was carried out to identify the vitamin B12 level on which jaundice occurred in 154 cases including the patient and control groups. Based on the analysis, the vitamin B12 level cutoff value was

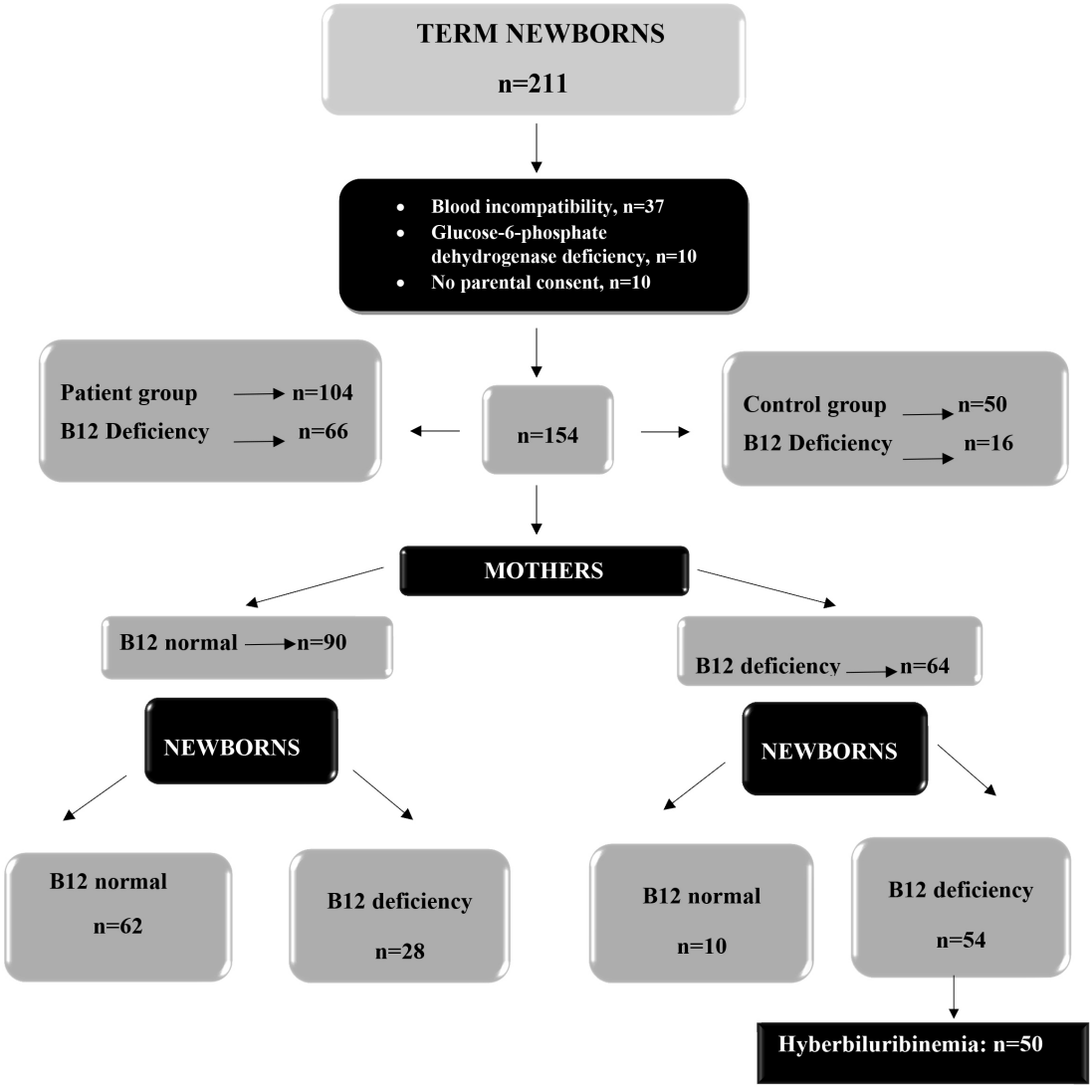

Figure 1. Mothers and newborns distribution.

218 ng/L (p<0.001). There was 53% sensitivity and 76.4% specificity for this value (Table 3).

Accepting that the vitamin B12 level cutoff value was 218 ng/L, the vitamin B12 deficiency in the patient group was again found to be significantly lower (Table 4).

The median median vitamin B12 level was 620 ng/mL in the 1st month after treatment in the patients with vitamin B12 deficiency.

Discussion

NH is one of the most common problems in the neonatal period, and its etiology cannot be determined in most cases. It has been reported in studies conducted in Turkey that the cause of NH could not be determined at a rate of up to 66-78% (10,11). Early diagnosis and treatment may be achieved by conducting etiology studies to prevent possible neurological problems.

Since vitamin B12 plays a role in maturation of erythrocytes and DNA synthesis, it is known that its deficiency leads to an increase in erythrocyte destruction due to ineffective erythropoiesis and hyperbilirubinemia. The studies investigating its frequency in newborns, its role in etiology in NH or its effects on the severity of the disease are very limited. Considering that vitamin B12 deficiency detected in infants based on newborn screenings may be mainly due to their mothers, it is very important that mothers and newborns are assessed together, and the treatment plan is carried out together (12).

In this prospective study, the vitamin B12 levels of mothers and their babies were assessed together in NH, and it was found that vitamin B12 deficiency

Table 2. Comparison of the patient group with normal and low vitamin B12 levels

	Normal vitamin B12 n=37	Low vitamin B12 n=67	p
Maternal age (years), mean ± SD	29.4±4.6	28.2±5.6	0.27
Male, n (%)	16 (43.2)	31 (46.1)	0.4
Gestational week, mean ± SD	38.2±0.8	38.2±1.0	0.1
Birth weight (g), mean ± SD	3202±488	3364±424	0.78
Cesarean, n (%)	25 (67.6)	38 (56.7)	0.1
APGAR 1, median (min-max)	9 (8-10)	9 (9-10)	0.1
APGAR 5, median (min-max)	9 (9-10)	9 (9-10)	0.1
Age of admission (days), mean ± SD	5.3±2.2	4.9±1.8	0.34
Total bilirubin (mg/dL), mean ± SD	13.3±2.6	14.4±2.7	**0.04**
Total bilirubin >15 mg/dL, n (%)	5 (13.5)	22 (32.8)	**0.02**
Hemoglobin (g/dL), infant, mean ± SD	16.3±3.1	15.7±2.6	0.54
Reticulocyte count (%), mean ± SD	1.7±1.2	1.4±0.9	0.24
Vitamin B12 level (ng/L), infant, mean ± SD	402±155	179±79	**0.0001**
Vitamin B12 deficiency, mother n (%)	4 (10.8)	46 (68.6)	**0.0001**

Table 3. Values determined as a result of ROC analysis

	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
Vitamin B12 level ≤218 ng/L	53.06	76.47	81.2	45.9

Table 4. Comparison of vitamin B12 deficiency in the patient and control groups according to the new cut-off value

Cut-off=218 ng/L	Patient group n=104	Control group n=50	p
Vitamin B12 deficiency, infant n (%)	58 (55.8)	12 (24)	**0.0001**
Vitamin B12 deficiency, mother n (%)	41 (39.4)	7 (14)	**0.003**

played an important role in the etiology of NH, and deficiency in the infants originated from their mothers.

Finkelstein et al. (6) investigated vitamin B12 levels in pregnant adolescents and their babies, and they found that the vitamin B12 levels of the mothers fell during pregnancy, and this was associated with the vitamin B12 levels of their babies. It was also demonstrated in their study that vitamin B12 levels decreased further as pregnancy progressed. Visentin et al. (7) reported that vitamin B12 deficiency increased further at the late stages of pregnancy, in a similar way, in their study on vitamin B12 levels in Canadian pregnant women. Hay et al. (8) showed that maternal vitamin B12 levels are a strong predictor of infants' blood vitamin B12 levels at birth. They also demonstrated that vitamin B12 supplements used in pregnancy increase umbilical cord blood vitamin B12 levels. Çoban et al. (13) reported that maternal vitamin B12 deficiency is the major cause of vitamin B12 deficiency in newborns, by revealing that the positive correlation between vitamin B12 levels of mothers and newborns is significant.

Onal et al. (10) noted that inadequate animal protein consumption due to low socioeconomic status in pregnancy is an important risk factor for vitamin B12 deficiency in both mothers and newborns. They emphasized the need for the use of parenteral vitamin B12 during pregnancy in developing countries such as Turkey.

In our study, as well, 84.4% of the mothers of the newborns with low vitamin B12 were found to have vitamin B12 deficiency, which was significantly different in comparison to the mothers of the newborns with normal vitamin B12 levels.

Despite the information in the literature on deficiency of vitamin B12 in the mother causing deficiency in the infant, there are no large-scale case studies that explore the role of this condition in NH.

In the only study reported from Turkey, Eroglu et al. (14) found that vitamin B12 deficiency was significantly higher in cases of prolonged jaundice involving 20 patients than in the control group. However, in their study, maternal vitamin B12 levels were not checked (14). In our study, a total of 154 mothers and their newborns were assessed and significantly higher vitamin B12 deficiency was detected in the patient group.

In our study, only babies who were breastfed were included. Vitamin B12 levels have also been found to be low in the milk of mothers with vitamin B12 deficiency in previous studies. Specker et al. (15) found that vitamin B12 was low in the milk of mothers with vitamin B12 deficiency.

The NH patients with normal and low levels of vitamin B12 were found to be similar in terms of the demographic characteristics. The mean bilirubin levels and need for phototherapy were significantly higher in with vitamin B12 deficiency patient. In the only study investigating the relationship between jaundice and vitamin B12 deficiency in the literature, neither the severity of the disease nor the need for treatment was taken into account.

Numerous different cutoff values for vitamin B12 levels have been determined in previous studies. In a study on neonatal cases with hyperbilirubinemia in India, Sukla et al. (16) determined the vitamin B12 cutoff value as 201 pg/mL. In a study by Koc et al. (17) in Şanlıurfa in Turkey, the vitamin B12 cut-off value was determined to be 207 pg/mL. In a study by Hay et al. (8) in Norway, the limit for vitamin B12 levels was determined to be 404 pg/mL.

The reference value in our study was taken to be 250 pg/mL in line with the guidelines (5). However, when an ROC analysis was carried out through our own cases, the cutoff value was determined to be 218 ng/L (1 ng/L=1 pg/mL). There was no difference in the results of the study when the patients were reassessed based on this value.

Conclusion

This study has demonstrated that vitamin B12 deficiency in mothers is related to vitamin B12 deficiency in newborns, and vitamin B12 deficiency in newborns significantly leads to hyperbilirubinemia.

It has been shown that, in NH cases for whom the etiology cannot be determined, vitamin B12 deficiency may have a role at a rate as high as 60%, which demonstrates the importance of assessing vitamin B12 levels in mothers and newborns in the early period.

Ethics

Ethics Committee Approval: The study protocol was approved by the Uludağ University Ethics Committee on the decision date of 11 January 2018 date and with the decision number of 2018-1/13.

Conflict of Interest: No conflict of interest was declared by the authors.

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