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An Implicit Cause of Prolonged Neonatal Jaundice: Vitamin B12 Deficiency

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Abstract

Objective Prolonged jaundice is defined as a serum bilirubin level of more than 5 mg/dL, which persists at postnatal 14 days in term infants and 21 days following birth in preterm infants. Although the underlying causes cannot be found in the majority of prolonged jaundice cases, this may be the first sign of a serious issue. Therefore, this study aimed to evaluate the association between vitamin B12 deficiency and prolonged jaundice in newborns.

Material and Methods This descriptive cross-sectional study was performed in a university hospital between January 1, 2015 and October 1, 2020. All participants consisted of infants who were admitted to the pediatric outpatient clinics. Infants >35 weeks of gestation and with prolonged jaundice of unknown etiology were included in the study group. The control group consisted of infants >35 weeks of gestation without prolonged jaundice. Demographic and clinical characteristics and serum vitamin B12 levels were evaluated comparatively.

Results A total of 126 infants, 66 of whom had prolonged jaundice, were included. The mean gestational week of the study group was 38.4 ± 1.8, and the control group was 38.6 ± 1.9 weeks. There was no difference between the groups in terms of demographics and laboratory data. The vitamin B12 level of the study group was significantly lower (median = 170 pg/mL) than the control (median = 268 pg/mL).

Conclusion Based on the findings of this study, vitamin B12 deficiency was thought to be an important cause of prolonged jaundice, and further studies are needed to explain the role of vitamin B12 deficiency in the etiology of prolonged jaundice.

Keywords
- prolonged jaundice
- vitamin B 12 deficiency
- newborn

Introduction

Prolonged jaundice is referred to as a serum bilirubin level of more than 5 mg/dL that persisted beyond the postnatal 14th day in term infants or the postnatal 21st day for preterm infants.[1,2] In the postnatal first week, almost half of the newborns develop hyperbilirubinemia, and in 15 to 40% of breastfed infants, it lasts more than 14 days.[3]

The etiology of prolonged jaundice should be elucidated cautiously. Prolonged unconjugated hyperbilirubinemia can be caused by particular entities including hemolytic diseases

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(Rh or AB0 incompatibility, or glucose-6-phosphate dehydrogenase [G6PD] deficiency), congenital hypothyroidism, urinary tract infection, and Crigler–Najjar or Gilbert syndromes.[4,5] Although breastfeeding-associated hyperbilirubinemia is the most common reason for prolonged jaundice, the underlying mechanism has not been explained completely yet.[6,7] Laboratory studies are not always adequate to determine the exact reason for prolonged jaundice, so this emerges as an issue for parents and physicians. Breast milk jaundice stands as a diagnosis of exclusion following the whole workup for the other etiologic factors.[1]

Vitamin B12 deficiency plays a role in certain hematological and neurological diseases.[8] Ineffective erythropoiesis due to vitamin B12 deficiency goes to hemolysis. Hence, lysis of erythrocytes elevates plasma bilirubin levels that can end in jaundice in newborns.[9] Depending on this fact, vitamin B12 deficiency can be considered one of the implicit reasons for prolonged jaundice. Therefore, this study aimed to evaluate the association between vitamin B12 deficiency and prolonged jaundice of newborns.

Methods

This descriptive cross-sectional study was performed in a university hospital's neonatal and pediatric outpatient clinic between January 1, 2015 and October 1, 2020. The local ethics committee of the study center approved the study (Ethics Committee approval number: KAEK/2020/162). Informed consent was obtained from parents. All cases consisted of infants who were admitted to the outpatient clinic for routine control. The study group comprised 66 infants who were born after 35 weeks of gestation with prolonged jaundice of undetermined etiology. The control group comprised 60 infants who were born after 35 weeks of gestation without prolonged jaundice. Serum vitamin B12 levels were examined in both groups from 14 to 60 days of age.

Serum vitamin B12 level was measured using the electrochemiluminescence immunoassay method using a Roche Cobas e-801 device.[10] Serum bilirubin levels were measured using the colorimetric diazo method using a Roche Cobas c501 device.[11] Prolonged jaundice diagnosis was determined by identifying serum total bilirubin levels above 5 mg/dL on the 14th day or later in term infants and the 21st day or later in preterm infants.[1]

The reference interval for serum vitamin B12 levels varies by country and region.[12] In this study, vitamin B12 level was classified as follows: low, < 232.5 pg/mL; normal, ≥ 232.5 pg/mL.[12,13] Vitamin B12 replacement was applied to all infants with vitamin B12 deficiency, with or without clinical findings. Infants who were exclusively breastfed were included in the study. Infants who received formula and/or less breast milk were excluded. Blood types of the mother and infant, direct Coombs test, reticulocyte count, total bilirubin value at the time of extended jaundice diagnosis, whole blood count, presence of reducing substance in urine, G6PD activity, and thyroid function tests (thyroid-stimulating hormone and free thyroxine levels) were recorded. The biochemical parameters of serum aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, and urine tests were investigated. Abdominal ultrasonography was performed to assess the liver and biliary tract. Those with a gestation age below 35 weeks, life-threatening acute problems, requiring neonatal intensive care, chromosomal or congenital anomalies, and a direct bilirubin level higher than 20% total bilirubin or higher than 2 mg/dL were excluded. Cases where prolonged jaundice's etiologies were determined (Rh or AB0 incompatibility, G6PD deficiency, galactosemia, enzyme deficiency, congenital hypothyroidism, urinary tract infection, cephalic hematoma, surrenal hemorrhage, or cholestasis) were excluded. Those with consanguinity, a family history of anemia, and/or hematological diseases were excluded from the study. The antenatal, natal, and postnatal features of the newborns were recorded. The prenatal history of the mother was assessed including preeclampsia, eclampsia, hypertension, and hypothyroidism. Birth history included date of birth, birth weight, gestational age, sex, type of birth, early membrane rupture, and the presence of meconium-stained amniotic fluid. Family features included several siblings and jaundice history in siblings. Postnatal information included the day of jaundice onset, whether phototherapy was received, the presence of cephalic hematoma on physical examination, admission to the hospital, and ingredients of feeding. Treatments administered during clinical follow-up were determined.

Data Analysis

The IBM SPSS statistics 21.0 program was used for statistical analysis. The Shapiro–Wilk test was used to assess whether variables showed normal distribution. Variables determined not to show normal distribution used median values for descriptive statistical analyses, while variables with normal distribution used mean $\pm$ standard deviation. Comparisons between the groups used the chi-square test for categorical data. For nonparametric paired comparisons, Mann–Whitney U test was used. A p-value of ≤ 0.05 was considered statistically significant.

Results

The demographic characteristics of 66 infants with prolonged jaundice and 60 infants without jaundice as the control group are presented in ►**Table 1**. There was no difference between the demographic and laboratory data of the two groups, as shown in ►**Table 1**. There was no significant difference between the two groups for age, gender, delivery method, gestational age, birth weight, breastfeeding, and levels of hemoglobin, platelets, or leukocytes (all p-values > 0.05).

The median vitamin B12 level of the patient group (group 1) was 170 (min: 86, max: 617) pg/mL and the control group (group 2) was 268 (min: 148, max: 585) pg/mL as shown in ►**Table 2**. The vitamin B12 level of group 1 was significantly lower than group 2 ($p < 0.001$) (►**Fig. 1**). A total of 20 infants (33.3%) did not have any pathological physical examination findings in the control group. The most common reason to measure the level of vitamin B12 was maternal B12 deficiency

Table 1 Demographic and clinical characteristics of group 1 and group 2

	Group 1	Group 2	
	Infants with prolonged jaundice	Infants w/o prolonged jaundice	
	Mean ± SD	Mean ± SD	*p*-Value
	N (%)	*N* (%)	
Age at serum collection/d	22.7 ± 8.5	24.5 ± 7.8	0.68
Gender			0.86
Male	34 (51.5)	31 (51.6)	
Female	32 (48.4)	29 (48.3)	
Delivery type			0.36
Vaginal	30 (45.4)	27 (45)	
C-section	36 (54.5)	33 (55)	
Gestational age (wk)	38.4 ± 1.8	38.6 ± 1.9	0.28
Birth weight (g)	3244 ± 508	3280 ± 420	0.12
Hemoglobin, g/dL	14 ± 2.4	15 ± 0.4	0.66
Leukocyte, /mm^3	9600 ± 2400	9800 ± 2500	0.74
Platelet, /mm^3	290.100 ± 90.000	302.100 ± 84.000	0.70
Total bilirubin, mg/dL	8.4 ± 4.2	–	

Abbreviations: C-section, Cesarean section; SD, standard deviation.

Table 2 Comparison of vitamin B12 levels groups

	Group 1	Group 2	*p*-Value
	Median (Min–Max)	Median (Min–Max)	
Vitamin B12, ng/L	170 (86–617)	268 (148–585)	< 0.001

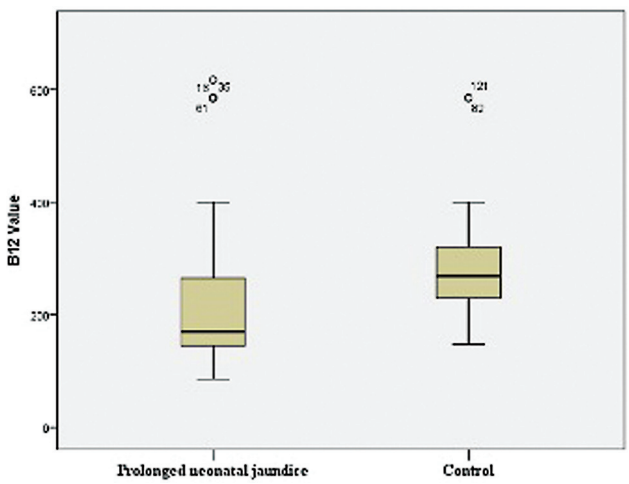

Fig. 1 Vitamin B12 (pg/mL) levels of prolonged neonatal jaundice and the control group.

(26.6%). Other reasons were anemia (13.3%), weak smiling (11.6%), hypotonicity (8.3%), lack of head control (5%), and uncoordinated eye movements (1.6%) (►**Table 3**).

Among infants in group 1, 21.2% had anemia, 14.8% had hypotonicity, 10.6% were in lack of head control, and 53.1% had no clinical findings (►**Table 3**). Forty-seven infants with prolonged jaundice and 17 infants of the control group had vitamin B12 deficiency. A comparison of the vitamin B12 deficient prolonged jaundice group and the control group can be seen in ►**Fig. 2**.

The mean vitamin B12 level of the prolonged jaundice group with vitamin B12 deficiency was 147.4 ± 5.13 pg/mL versus 201.1 ± 6.12 pg/mL in the control group with vitamin B12 deficiency as shown in ►**Fig. 2**. The vitamin B12 level of the prolonged jaundice group with vitamin B12 deficiency was significantly lower than the control group with vitamin B12 deficiency ($p < 0.001$).

Discussion

Many studies explained that most of the prolonged jaundice cases with undetermined etiology were related to breast milk jaundice.[8,12,14] To the best of our knowledge, this is the first study showing an association between prolonged jaundice and vitamin B12 deficiency. Aziz and Elela demonstrated that the vitamin B12 level of newborns with jaundice was significantly lower than healthy controls.[9] Maternal vitamin B12 is positively correlated with neonatal vitamin B12 and negatively correlated with neonatal total serum bilirubin levels.[9]

Table 3 Admission complaints of participants in the group with and without prolonged jaundice

Participants without prolonged jaundice	*n*	%
Anemia	8	13.3
Hypotonicity	5	8.3
Lack of head control	3	5
Uncoordinated eye movements	1	1.6
Weak smiling	7	11.6
Maternal vitamin B12 deficiency	16	26.6
Prolonged jaundice group with vitamin B12 deficiency		
Anemia	10	21.2
Hypotonicity	7	14.8
Lack of head control	5	10.6
Without clinical findings	25	53.1

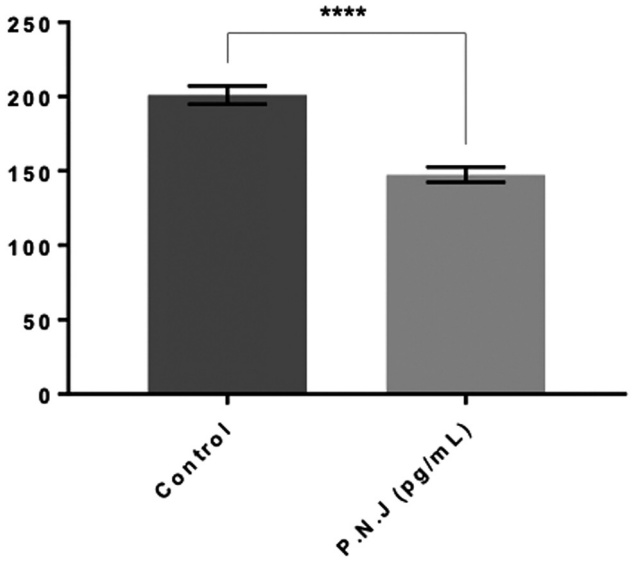

Fig. 2 Comparison of vitamin B12 (pg/mL) deficient prolonged jaundice group and control group.

In the present study, serum vitamin B12 levels in infants with prolonged jaundice and undetermined etiology were significantly lower in the study group. Ineffective erythropoiesis results from intramedullary apoptosis of megaloblastic erythroid precursors. The erythrocytes, which were produced with abnormal cellular membrane proteins, have increased stiffness leading to shortened red blood cell survival. As a result of this ineffective erythropoiesis, jaundice develops with increased plasma bilirubin level.[9]

In the literature, there are no studies showing correlations between vitamin B12 deficiency with other causes blamed for breast milk jaundice such as β-glucuronidase, free fatty acids, pregnan-3a, and genetic causes.[3] Vitamin B12 deficiency causes much more significant neurological symptoms in infants who have rapid growth like in the newborn period, but anemia-associated findings are more common in other periods. Children with insufficient vitamin B12 stores have normal development in the first months following birth. Among these, 70% develop clinical findings at 3 to 6 months of age.[15] The most common findings are lethargy, hypotonia, and convulsions. Some infants may even be found in a comatose state.[16] Neurological symptoms may ameliorate in the first 3 to 4 months following treatment, may continue, or previous neurological findings may be worsened. Brain atrophy or hypoplasia may be observed in patients. Most patients complete their brain myelinization within 12 to 13 months following treatment; however, cranial magnetic resonance imaging shows that demyelination findings may continue for up to 3 years of life. Therefore, early diagnosis and treatment of vitamin B12 deficiency are crucial for the infant's neurologic development.[17–20]

Forty-seven infants with prolonged jaundice and 17 infants of the control group had vitamin B12 deficiency. The vitamin B12 level of the infants with vitamin B12 deficiency in the prolonged jaundice group was significantly lower than the control group infants with vitamin B12 deficiency (►**Table 2**). There was a statistically significant difference in vitamin B12 levels between the study groups. Hypotonicity was the most common clinical finding in vitamin B12 deficient infants in both groups (►**Table 3**). We believe that early detection and treatment of the deficiency prevent other possible neurological problems.

The study by Lai et al compared the cognitive scores of children at 2 years of age monitored in two groups whose mothers were with or without vitamin B12 deficiency in pregnancy. They identified that the cognitive scores of infants whose mothers experienced vitamin B12 deficiency were significantly lower than the infants whose mothers did not have vitamin B12 deficiency in pregnancy.[17] In the first 6 months of the infant age group, the only source of nutrition for infants is breast milk. Thus, the vitamin B12 level of the mother affects the vitamin B12 level in breast milk and the infant during the breastfeeding period.[21–23] In our study, all infants with prolonged jaundice only received breast milk. Whether vitamin B12 deficiency is screened during

pregnancy, it could be identified and treated. Thus, possible anemia or other issues linked to vitamin B12 deficiency and significant neurological delay could be prevented.

A limitation of our study is that the vitamin B12 levels in the mothers and serum homocysteine and methylmalonic acid levels of the infants were not included due to the retrospective design of the study. Serum homocysteine and methylmalonic acid levels are sensitive markers of vitamin B12 deficiency.[8,24] Other limitations of this study are as follows. Vitamin B12 causes ineffective erythropoiesis and is considered a cause of jaundice. However, since there was no difference between the groups in terms of anemia, the authors did not include parameters such as reticulocyte mean corpuscular volume which are markers in this regard.

Conclusion

Depending on this study, vitamin B12 deficiency is found to be associated with prolonged jaundice, and a delayed diagnosis can cause significant neurodevelopmental issues in infants. Therefore, further studies are needed to elucidate the cause and effect mechanisms underlying vitamin B12 deficiency and prolonged jaundice.

Conflict of Interest
None declared.

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