Vitamin B12 Serum Levels of Six to Nine-month-old Infants According to Feeding Practices

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ABSTRACT

Aim: Vitamin B12 is naturally obtained from animal-derived foods and is important for the development and wellbeing of babies. Vitamin B12 deficiency is a general health concern in developing countries. This study aimed to investigate the effects of different feeding practices on vitamin B12 levels and hemogram parameters in infants aged 6-9 months.

Materials and Methods: A total of 120 infants (61 boys, 59 girls) aged 6-9 months were retrospectively evaluated. These babies were assigned to three groups according to feeding practices: 1) only breast milk, 2) breast milk and formula, and 3) only formula in addition to complementary foods. Laboratory data (vitamin B12, hemoglobin, hematocrit, mean corpuscular volume, white blood cell count, and absolute neutrophil count) of the patients were retrospectively compared.

Results: The mean and median age of the patients was 6.6±1.1 months. The vitamin B12 levels in babies that only received formula were statistically significantly higher than those in babies taking only breast milk or taking both breast milk and formula (p<0.05). Furthermore, vitamin B12 levels were significantly lower in babies receiving only breast milk than in those receiving both formula and breast milk (p<0.001).

Conclusion: This study showed that vitamin B12 levels were lower in babies that only received breast milk compared to those in babies receiving formula. The babies that were receiving formula only had the highest levels of vitamin B12. This study determined that breast milk alone was not sufficient to maintain normal levels of vitamin B12 in 6 to 9-month-old babies in the region. Therefore, both mothers and babies should be provided with the necessary support in terms of vitamin B12 levels, and prophylaxis should be discussed from the viewpoint of preventive healthcare services.

Keywords: Child, breast milk, formula, vitamin B12

Introduction

The most significant role of vitamin B12 is its function in DNA synthesis, which is required for cell division and proliferation. Vitamin B12 is water-soluble and synthesized mainly by microorganisms. Humans cannot synthesize vitamin B12. Vitamin B12 is obtained from cobalamin in foods, and especially in animal-derived foods. Dietary deficiency of this vitamin is rare for those who have normal eating habits, as the amount of vitamin B12 is sufficient in most animal-obtained foods. However, vitamin B12 inadequacy is seen in those with a low dietary intake of vitamin B12 (1).

Vitamin B12 is a significant factor for the process of child development. Vitamin B12 deficiency can cause neurological disorders and hematologic changes, in addition...
to developmental delays and regressions (1). Vitamin B12 fortified foods and supplements, in addition to animal-derived foods, can be consumed in cases of deficiency (2). Vitamin B12 inadequacy is prevalent in those on strict vegetarian or vegan regimes as well as in individuals who live in developing countries (3-6).

Breast milk is the main source of micronutrients needed by a newborn (7), and babies who are only fed with breast milk receive nearly 0.25 µg vitamin B12 from a healthy mother within the first 6 months (4). The vitamin B12 level of babies is associated with the vitamin B12 level of their mothers (8). Previous studies have shown that babies receiving breast milk alone had notably lower serum vitamin B12 rates in comparison to those babies receiving formula (9,10).

Delayed diagnosis and treatment during childhood, in spite of the low cost of this treatment, may entail irreversible neurological damage, in addition to severe anemia. Therefore, patients presenting to an outpatient clinic should be examined for vitamin B12 inadequacy. Anemia within the first 2 years of life can occur due to a vitamin B12 deficiency in breastmilk, especially in developing countries, and manifests with neurodevelopmental delay (11,12).

This research aimed at investigating the effectiveness of varied feeding practices on vitamin B12 serum levels and hemogram parameters in 6 to 9-month-old infants.

**Materials and Methods**

The Clinical Trials Ethics Committee of Okmeydanı Training and Research Hospital approved this study as per the approval number:1079, date: December 18th, 2018. Consent forms were filled out by all participants. The study groups consisted of 120 babies aged 6-9 months, consisting of 61 males and 59 females who did not have any medical problems and were brought to the pediatric outpatient clinics of Okmeydanı Training and Research Hospital between January 2018 and November 2018 for routine checkup and immunization follow-up. Data on feeding history and multivitamin use were obtained for all infants. Babies who were premature, had a low birth weight, had any metabolic or chronic disease, or were hospitalized for any disease were excluded from this study. Patients who had incomplete files, or those who did not have data concerning B12 levels or complete blood count data, or those with an indeterminable feeding history were not included in this study.

The infants were divided into three groups according to their feeding practices: 1) only breast milk, 2) breast milk and formula, and 3) only formula in addition to complementary foods. In this study, 77 babies received only breast milk, 19 babies received both breast milk and formula, and 24 babies received formula only. In addition, all babies were receiving complementary foods according to the statements of their families. However, objective data on the amount of complementary foods given to the baby could not be obtained. Laboratory datum [vitamin B12, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), white blood cell count (WBC), and absolute neutrophil count (ANC)] of the patients were retrospectively evaluated between the groups. Vitamin B12 levels were assayed using the chemiluminescent immunoassay with a Roche Cobas Integra 400 Plus analyzer. Values lower than 250 pg/mL were considered low. Hemogram parameters were assayed with a Mindray BC-6800 hemogram device.

**Statistical Analysis**

Data were analyzed using the SPSS 20.0 for Windows package software (IBM). Continuous variable data with a normal distribution are given as mean ± standard deviation, and data with a non-normal distribution are given as median and range. Data with a normal distribution was analyzed using a histogram and Kolmogorov-Smirnov test. The statistical differences between non-normal data were analyzed using the Mann-Whitney U test, while the differences between more than two groups were analyzed using the Kruskal-Wallis test. The statistical differences between two categorical datasets were analyzed using the chi-square test. Categorical data are stated numerically with percentage. P<0.05 was acknowledged as statistically meaningful.

**Results**

The mean and median age of the patients was 6.6±1.1 months; 50.8% (n=61) of the patients were male and 49.1% (n=59) of the patients were female. Vitamin B12 levels in those infants that only received formula were statistically notably higher than those in infants receiving only breast milk or both breast milk and formula (p<0.05). Vitamin B12 levels were significantly lower in infants receiving only breast milk than in those receiving formula in addition to breast milk (p<0.05). There was no statistically notable difference between the groups in terms of Hb, Hct, MCV, WBC, and ANC levels (Table 1).
Vitamin B12 deficiency was mainly seen in babies receiving only breast milk. None of the babies receiving only formula had vitamin B12 deficiency (Table 2).

The mean baby weight, gender, type of birth (normal birth or cesarean section) and gestational weeks of the three groups were found to be statistically similar (Table 3).

**Discussion**

Vitamin B12 deficiency is a health worry generally encountered in developing countries (5). Vitamin B12 is critical for development during the fetal, neonatal, and infancy periods.

Therefore, vitamin B12 deficiency can cause numerous diseases, especially developmental and neurological disorders (13,14). It is important for a mother to consume foods that are rich in vitamin B12 throughout the lactation period (15). It is recommended to breastfeed babies for more than 6 months, and breast milk is a natural source of vitamin B12 for the baby. However, some studies have shown that restrictive vegetarian and vegan diets resulted in lower levels of vitamin B12 in the mother and therefore led to vitamin B12 deficiency in babies (4,15-18). Vitamin B12 deficiency is not uncommon in babies receiving only breast milk (4,17-21). Studies have shown that babies who were fed formula had increased vitamin B12 levels compared to those that were breast fed (9,10).

In this study, the effects of varied feeding implementations on vitamin B12 levels in the bloodstream of babies who were brought to our hospital were investigated. Similar to other studies, this current study revealed that vitamin B12 levels were lower in infants receiving only breast milk compared to those receiving formula in addition to breast milk as well as those receiving only formula. It was also observed that those babies who were only receiving formula had much higher levels of vitamin B12. According to these results, it was apparent that vitamin B12 levels differed according to feeding practices. It is therefore important to consume formulas fortified with vitamin B12 to prevent vitamin B12 deficiency.

Vitamin B12 insufficiency in mothers is the leading reason of vitamin B12 deficiency in babies during infancy. When babies are only fed breast milk, they can have severe vitamin B12 deficiency if their mothers also have vitamin B12 deficiency. (22,23). In a study conducted in Istanbul and Izmit, Ackurt reported that 48% of women in the early pregnancy period (13-17 weeks), 80% of women in the late pregnancy period (28-32 weeks), and 60% of women in the postpartum period had vitamin B12 deficiency. In addition, the delayed or non-introduction of complementary feeding or feeding babies with cow's milk as the primary food source contributed to vitamin B12 insufficiency in infants (24). In

**Table I. Biochemical data of the groups according to feeding practices**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Babies receiving breast milk only (n=77) (mean ± SD)</th>
<th>Babies receiving breast milk + formula (n=19) (mean ± SD)</th>
<th>Babies receiving formula only (n=24) (mean ± SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>228.8±155.5</td>
<td>359.5±192.9</td>
<td>618.5±248.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.5±1.0</td>
<td>11.6±0.8</td>
<td>11.9±1.0</td>
<td>0.404</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>33.8±3.2</td>
<td>33.0±2.5</td>
<td>34.1±3.0</td>
<td>0.295</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>90.6±106.5</td>
<td>73.4±3.3</td>
<td>74.9±4.3</td>
<td>0.380</td>
</tr>
<tr>
<td>WBC (mm³)</td>
<td>9.549±2.544</td>
<td>10.248±2.568</td>
<td>8.655±1.541</td>
<td>0.194</td>
</tr>
<tr>
<td>ANC (mm³)</td>
<td>2.224±1.465</td>
<td>1.762±2.714</td>
<td>1.989±2.714</td>
<td>0.400</td>
</tr>
</tbody>
</table>


**Table II. Evaluation of vitamin B12 levels according to the reference interval in different feeding practices**

<table>
<thead>
<tr>
<th></th>
<th>Babies receiving only breast milk (n=77)</th>
<th>Babies receiving breast milk and formula (n=19)</th>
<th>Babies receiving only formula (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 below the reference interval</td>
<td>37 (48.1%)</td>
<td>3 (15.8%)</td>
<td>0-0</td>
</tr>
<tr>
<td>Vitamin B12 within the reference interval</td>
<td>40 (51.9%)</td>
<td>16 (84.2%)</td>
<td>24 (100%)</td>
</tr>
</tbody>
</table>

**Table III. Demographic features of babies**

<table>
<thead>
<tr>
<th></th>
<th>Babies receiving breast milk (n=77)</th>
<th>Babies receiving breast milk + formula (n=19)</th>
<th>Babies receiving formula only (n=24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby weight (gram)</td>
<td>3,520.2±199.5</td>
<td>3,466±121.2</td>
<td>3,320±124.2</td>
<td>0=0.124</td>
</tr>
<tr>
<td>Baby gender (male/female)</td>
<td>35/42</td>
<td>10/9</td>
<td>13/11</td>
<td>0=0.130</td>
</tr>
<tr>
<td>Delivery method (normal birth/cesarean section)</td>
<td>45/32</td>
<td>13/6</td>
<td>17/7</td>
<td>0=0.109</td>
</tr>
<tr>
<td>Gestational week (week)</td>
<td>39.1±0.4</td>
<td>39.3±0.8</td>
<td>38.9±0.7</td>
<td>0=0.095</td>
</tr>
</tbody>
</table>
another study conducted in Sivas, Demirel et al. (25) stated that the incidence of vitamin B12 insufficiency during the third trimester was 66.7% in healthy pregnant women.

In a study by Koç et al. (26), 39.8% of babies who were healthy according to their families had a vitamin B12 deficiency, and 75% of the mothers of those babies with vitamin B12 deficiency also had vitamin B12 insufficiency. Monagle et al. (27) found infantile megaloblastic anemia in 19 children under the age of 1 in their clinic, and reported that vitamin B12 insufficiency in 6 (30%) children was secondary to the vitamin B12 insufficiency in their mothers, and that these 6 children were only receiving breast milk. In another study, Minet et al. (28) demonstrated that healthy infants who were receiving breast milk had lower vitamin B12 levels than those who were receiving formula.

This current study’s findings are similar to the results of previous studies. It has been determined that, despite complementary feeding, breast milk alone was not adequate to sustain normal levels of vitamin B12 in 6 to 9-month-old infants in this region. Vitamin B12 levels, which can lead to negative outcomes in cases of deficiency, should be measured in infants (22,28). Vitamin B12 levels should also be assessed in mothers, and those with low levels of vitamin B12 should be provided with vitamin supplement in order to increase the vitamin B12 level in their breast milk. Accordingly, a vitamin B12 test and complete blood count should be performed when an infant is brought to an outpatient clinic. In this study, 3 babies had a vitamin B12 deficiency even though they were receiving formula in addition to breast milk. Consequently, formula intake cannot completely rule out vitamin B12 deficiency, since infants taking formula in addition to breast milk also had a vitamin B12 deficiency.

When clinical signs suggest vitamin B12 deficiency in a child, fasting plasma homocysteine levels should be investigated even if serum vitamin B12 level and MCV results are normal. It has been reported that testing only for vitamin B12 levels could lead to a misdiagnosis in 10-26% of patients. Accuracy can be increased to 99.8% when methyl malonic acid and homocysteine tests are carried out (29). Accurate diagnosis is of the utmost importance in these patients in order to administer vitamin B12 in addition to treatment to the infants receiving breast milk. Homocysteine levels were not measured in this study as none of the patients had neurodevelopmental disorders, according to their records. Moreover, we suggest that standard algorithms concerning an approach to vitamin B12 deficiency in children can be created as the number of studies on this field increases.

Previous studies have shown that vitamin B12 insufficiency can be caused by strict vegetarian diets or pernicious anemia for 90% of infants (27,30). On the other hand, determinants such as poverty or low socioeconomic status, incorrect feeding habits, and an increased use of vitamin B12 due to a high number of pregnancies also have an impact on the high percentage of vitamin B12 deficiency in babies and their mothers in underdeveloped and developing countries (31,32). It is also apparent that the risk of developing vitamin B12 deficiency increases with lower economic status and a higher number of pregnancies. In the current study, none of the babies receiving formula had a vitamin B12 deficiency. Therefore, infants should receive a sufficient amount of complementary foods rich in vitamin B12. Accordingly, it is important to consider and provide early treatment for vitamin B12 deficiency in breastfeeding mothers and their infants with a low intake of animal-derived foods, especially in regions with low socioeconomic status, in addition to a vegetarian diet.

Of the hematologic findings for vitamin B12 deficiency, increased MCV characteristically develops before anemia, and the clinical signs are observed at a later time. This study found that there was no statistically major variation between the groups with respect to MCV levels in healthy infants who had different feeding practices. However, the MCV levels in infants that only received breast milk were increased compared to those in the other groups.

It was observed that vitamin B12 levels were lower in infants taking only breast milk than in those taking formula, and that babies who were only taking formula had the highest levels of vitamin B12. This research confirmed that breast milk alone was not adequate to maintain normal levels of vitamin B12 in 6 to 9-month-old infants in the region. Accordingly, vitamin B12 levels should be checked during pregnancy and in case of deficiency, a suitable replacement therapy should be provided for pregnant women in a manner to encompass the postpartum period in order to protect the baby from the dramatic outcomes of vitamin B12 insufficiency. Vitamin B12 levels must be tested in addition to a complete blood count in routine screening of children. Formula should be recommended for babies found to have vitamin B12 deficiency to supplement insufficient breast milk from their mothers. Replacements should be administered if there is a vitamin B12 deficiency despite sufficient breast milk. In addition, routine B12 prophylaxis and dosages in infants should be discussed as a part of preventive medicine practices in developing countries.
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Ethics
Ethics Committee Approval: The Clinical Trials Ethics Committee of Okmeydanı Training and Research Hospital approved this study as per the approval number: 1079, date: December 18th, 2018.

Informed Consent: Consent forms were filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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