The Serum Concentrations of Trace Elements and Vitamin A in Turkish Six-Month-Old Infants with Different Feeding Practices

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Summary  Breast feeding is the first and most important step of a healthy diet. Breast milk contains important vitamins and trace elements such as iron, zinc, copper, and vitamin A. The aim of our study was to evaluate the levels of hemoglobin, hematocrit, mean corpuscular volume, serum iron, iron binding capacity, ferritin, serum zinc, copper and vitamin A in three groups of infants, which were determined based on feeding practices. The infants in all groups were not given prophylactic iron in the first 6 mo. Two hundred fifty-nine infants were included in the study. One hundred fifty-one (58.3%) were fed with breast milk, 91 (35.1%) were fed with breast milk+formula, and 17 (6.6%) were fed with formula only. Serum copper and vitamin A levels were found to be low in formula-only fed infants compared to other groups with a statistically significant difference (p=0.017, p=0.022 respectively). The serum zinc level was found to be low in 15.9% of the breast fed infants, 17.6% of the breast milk+formula fed infants, and 23.5% of the formula-only fed infants. Although the formula-only fed infants had lower values, the difference was not statistically significant among groups (p=0.716). We think that breast fed infants potentially have low levels of copper and vitamin A in the first 6 mo and may be offered supplements. Alternatively, formula mineral and vitamin contents could be enriched. We think that further studies on this subject are needed.

Key Words  breast milk, trace elements, vitamin A, infants

The importance of breast milk (BM) feeding in the first 6 mo of life is well regarded around the World. BM feeding in the first 6 mo decreases the frequency of many diseases, especially infectious diseases, and neonatal mortality rate while it supports appropriate nutrition, growth, and development. Even in the hottest climates, it is shown that BM fed infants do not require any additional nutrition, including water (1, 2).

Despite the known benefits of BM, the rate of BM feeding in Turkey is low. Based on the 2008 data on Turkey Population and Health Investigation, the rate of BM feeding was 97%, while the rate of BM only (BMO) feeding in the first 4–5 mo was reported as 41.6% (3).

BM composition has the capacity to provide all the nutrition needs of infants, except vitamin D in the first 6 mo. BM is digested far more easily than cow milk or milks formulated from cow milk. The absorption rate of minerals provided by BM such as iron and zinc in the intestines is much higher compared to other types of milk (1, 2).

Iron, zinc, and copper are important trace elements found in BM (1, 2). These trace elements have important roles in infants’ growth and development and immune system functions. Studies show that BM trace element content varies from country to country (4–7). There are studies that suggest the necessity of supplementing the low level trace elements in BM depending on geographic region (4, 5, 7). The trace element vitamin doses of formulas are determined for non BM-fed infants based on country regions. In Turkey, Ministry of Agriculture, Food and Livestock Regulation 98/20 defines allowed iron levels in formulas as min. 0.5 mg, max. 1.5 mg: zinc level as min. 0.5 mg, max. 1.5 mg; and copper level as min. 20 μg, max. 80 μg per 100 kcal (8).

Vitamin A also exists in BM in sufficient quantities. Its concentration in BM is 670 (retinol equivalent) μg/L (2). Ninety percent of vitamin A is stored in the liver, but 1% exists in serum, most of which is bound by retinol binding proteins. Newborns’ liver storage for vitamin A is insufficient and should be supplemented with BM (9). In Turkey, the permitted vitamin A level in formula is determined as min. 60 μg, max. 180 μg (retinol equivalent) (8).

The aim of our study was to evaluate the hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), serum iron, iron binding capacity (IBC), ferritin, serum zinc, copper and vitamin A levels in infants fed by BMO, BM+formula, or only formula. The infants included in this study were not given prophylactic iron in the first 6 mo.

MATERIAL–METHOD

Six-month-old infants who were carried to term and who did not receive phototherapy or blood change for
neonatal jaundice were considered in this study. The infants were followed in the Ankara Research and Training Hospital Pediatric Polyclinic and Healthy Child Polyclinic between January 2012 and December 2012. Pre-term infants, infants with a chronic illness, and infants who were given cow milk or iron supplements were not included in the study. All the infants were using vitamin D regularly.

Data on infants’ birth weights, sixth-month weights, mothers’ education level, number of pregnancies, and number of siblings were recorded. Blood samples from infants were taken for obtaining data on total blood count, serum iron, IBC, ferritin, serum zinc, copper and vitamin A. Infants were separated into three groups based on how they were fed: BMO fed, BM+formula fed, and only formula fed.

Based on laboratory results, the findings of Hb < 11.5 g/dL, Hct < %35, MCV < 70 fL/dL and ferritin level < 7 ng/mL were considered as iron deficiency anemia (IDA). Serum iron < 22 μg, serum zinc level < 9.8 μmol/L, serum copper level < 12.6 μmol/L, and serum vitamin A level < 0.7 μmol/L were considered significantly low (10, 11).

Total blood counts were obtained with Coulter LH 780 (Beckman Coulter, New York, NY) hemogram devices by reading EDTA–blood mix. Vitamin A levels were obtained by the high performance liquid chromatography (HPLC) method (Agilent 1100, Waldbronn, Germany; kit Chromsystems, Bremen, Germany) and zinc, iron, and copper were studied by the immunoturbid photometric method at 570–700 wavelength (Olympus AU 2700, Tokyo, Japan; kit Randox, London, UK).

The data of our study were evaluated with SPSS 15.0 for Windows software. Among the groups, data with good distribution (birth weight, sixth-month-old weight, Hb, MCV, and IBC) were quantified with means and standard deviation, while data with bad distribution (mother age, father’s age, number of pregnancies, number of siblings, Hct, ferritin, serum iron, zinc, copper, and vitamin A) were quantified with medians. The importance of difference among groups based on means was investigated with Student’s t and ANOVA tests, while the importance of difference based on medians was investigated with Mann-Whitney U and Kruskal-Wallis tests. The chi-squared test was used for comparing qualitative data. For p<0.05, the results were considered statistically significant.

All procedures performed in this study were in accordance with the ethical standards of the institutional and research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For the study, ethics committee approval number 3726 and dated 28.12.2011 from Ankara Research and Training Hospital and informed consent forms from families were obtained.

FINDINGS

Two hundred ninety-four infants 6 mo or older were evaluated for the study. Two hundred fifty-nine infants met the requirements to be included in this study, of which 151 (58.13%) were fed with BMO, 91 (35.1%) were fed with BM+formula, and 17 (6.6%) were fed with only formula. Mothers’ age median was 26 (18–52), fathers’ age median was 31 (21–52). Table 1 shows the demographic comparison of infants based on feeding groups. No statistically significant relation among mothers’ education level, mothers’ age, fathers’ age, number of pregnancies, type of birth, sex or type of feeding was determined (p>0.05).

When infants’ respiratory infection and related hospitalization histories were investigated, 74 (28.6%) of the infants were found to have such history. When evaluated based on feeding types, 38 (25.2%) of the BMO fed infants, 33 (36.3%) of the BM+formula fed infants, and
Comparison of Trace Elements and Vitamin A in Infants

Table 2. Comparison of laboratory results by groups.

<table>
<thead>
<tr>
<th></th>
<th>BMO</th>
<th>BM+ formula</th>
<th>Formula</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (mean) (g)</td>
<td>7.682±0.999</td>
<td>7.932±1.058</td>
<td>7.705±1.438</td>
<td>0.195</td>
</tr>
<tr>
<td>Hb (mean) (g/dL)</td>
<td>11.4±0.9</td>
<td>11.3±0.8</td>
<td>11.5±0.9</td>
<td>0.690</td>
</tr>
<tr>
<td>Hct (median) (%)</td>
<td>34 (27–42)</td>
<td>34 (25–39)</td>
<td>34 (26–38)</td>
<td>0.328</td>
</tr>
<tr>
<td>MCV (mean) (fL)</td>
<td>76.5±6.7</td>
<td>75.2±5.7</td>
<td>76.0±9.4</td>
<td>0.262</td>
</tr>
<tr>
<td>Iron (median) (µg/dL)</td>
<td>38 (7–119)</td>
<td>32 (7–119)</td>
<td>44 (15–83)</td>
<td>0.071</td>
</tr>
<tr>
<td>IBC (mean)</td>
<td>309.4±93.8</td>
<td>322.5±86.9</td>
<td>298.6±87.8</td>
<td>0.441</td>
</tr>
<tr>
<td>Ferritin (median) (ng/mL)</td>
<td>44 (6–182)</td>
<td>37 (3–150)</td>
<td>32 (8–108)</td>
<td>0.223</td>
</tr>
<tr>
<td>Copper (median) (µmol/L)</td>
<td>12.4 (4.8–27.0)</td>
<td>13 (5.0–36.4)</td>
<td>12.4 (8.0–18.9)</td>
<td>0.682</td>
</tr>
<tr>
<td>Zinc (median) (µmol/L)</td>
<td>20 (8–37)</td>
<td>20 (8–36)</td>
<td>15.3 (8.0–29.9)</td>
<td>0.041</td>
</tr>
<tr>
<td>Vitamin A (median) (µmol/L)</td>
<td>0.8 (0.1–2.3)</td>
<td>0.8 (0.2–2.9)</td>
<td>0.8 (0.2–1.4)</td>
<td>0.295</td>
</tr>
</tbody>
</table>

Table 3. Comparisons of the groups based on laboratory results.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>BMO</th>
<th>BM+ formula</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (&lt;11.5 g/dL)</td>
<td>138 (53.3)</td>
<td>84 (55.6)</td>
<td>45 (49.5)</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td>Hct (&lt;35)</td>
<td>170 (65.6)</td>
<td>102 (62.5)</td>
<td>58 (63.7)</td>
<td>10 (58.8)</td>
</tr>
<tr>
<td>MCV (&lt;70)</td>
<td>95 (36.7)</td>
<td>51 (33.8)</td>
<td>38 (41.8)</td>
<td>6 (55.3)</td>
</tr>
<tr>
<td>Fe (&lt;22)</td>
<td>51 (19.7)</td>
<td>24 (15.9)</td>
<td>24 (26.4)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>IBC (&lt;12.7)</td>
<td>33 (12.7)</td>
<td>18 (11.9)</td>
<td>13 (14.3)</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Ferritin (&lt;7)</td>
<td>2 (0.8)</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>0</td>
</tr>
<tr>
<td>Zn (&lt;9.8 µmol/L)</td>
<td>44 (17)</td>
<td>24 (15.9)</td>
<td>16 (17.6)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>Cu (&lt;12.6 µmol/L)</td>
<td>20 (7.7)</td>
<td>8 (5.3)</td>
<td>8 (8.8)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>vitamin A (&lt;0.7 µmol/L)</td>
<td>66 (25.5)</td>
<td>30 (19.90)</td>
<td>28 (30.8)</td>
<td>8 (47.1)</td>
</tr>
</tbody>
</table>

3 (17.6%) of the formula-only fed infants had hospitalization histories. There was no statistically significant difference among type of feeding, history of respiratory infection or history of hospitalization (p=0.106).

The birth weight mean was 3,198.5±375.3 g and sixth-month weight mean was 6,771.8±1,055.3 g. Laboratory results based on feeding groups are shown in Table 2. Table 3 shows the comparison of the lower than reference value laboratory results for the groups. No statistically significant difference based on IDA was determined among groups (p=0.647).

The serum zinc level was found to be low in 15.9% (n=24) of the BMO fed infants, 17.6% (n=16) of the BM+ formula fed infants, and 23.5% (n=4) of the formula-only fed infants. Though the formula-only fed infants had lower values, there was no statistically significant difference among groups (p=0.716) (Table 3). Serum copper and vitamin A levels were found to be low in formula-only fed infants compared to other groups with a statistically significant difference (p=0.017, p=0.022 respectively).

**DISCUSSION**

BM feeding is the first and most important step of a healthy diet for infants, because it contains essential trace elements and vitamins. One hundred milliliters BM includes 0.07 mg Fe, 0.04 mg Cu, 0.28 mg Zn and 670 (retinol equivalent) µg/L vitamin A (2, 12). In comparison, 100 kcal equivalent formula includes 0.5–1.5 mg Fe, 0.5–1.5 mg Zn, 20–80 µg Cu and 60–180 µg (retinol equivalent) vitamin A (8). Thanks to vitamins and trace elements in BM, infants do not need vitamin or mineral supplements in the first 6 mo (except vitamin D). Though studies show that especially iron, zinc, and copper content of BM is not affected by mothers’ diet, geographical variation is significant. Because of this, knowing whether or not trace elements in BM are taken sufficiently in the first 6 mo is important for determining if any trace element supplements are necessary for infants (4, 5, 7).

Iron content of the BM is independent of the iron levels of the mothers and their diet. However, fetal growth and iron storage of a newborn are related to mothers’ iron storage. Because of this, infants who have mothers with anemia are at risk based on IDA development (7, 13).

BM has a low iron level, but it has high absorptivity. In normal born infants with sufficient iron storage, IDA is not developed especially in the first 6 mo (13, 14). However, there are studies that report that BM does not meet infants’ iron need after 4 mo (15, 16). In their study, Vatandas et al. recommended that infants fed with BMO in the first 6 mo followed with mixed feeding should be given prophylactic iron from 4 mo to 1 y (17). In another study, no anemia was observed in infants who were fed with BMO. The same study found that 43% of...
infants who were fed with additional formula before seventh month had Hb<11 g/dL and it was suggested that infants fed with BMO are protected from anemia in the long term (18).

In our study, the BMO fed group had lower hemoglobin levels compared to formula fed groups; however, no statistically significant difference in IDA development was found. It was concluded that BM protects from anemia in the first 6 mo; prophylactic iron could be started from the sixth month. Similar to our study, Nadir et al. (19) did not find significant difference in average Hb levels among BMO fed children in the fourth and sixth months and those who did not take BM.

Zinc is one of the elements that have an important role in growth and development. Since 80% of the zinc in BM is absorbed, BM feeding in the first 6 mo is sufficient in terms of zinc supply. After 6 mo, BM cannot provide enough daily zinc and additional food rich in zinc (liver, meat, fish) should be given (20, 21). At the same time World Health Organization (WHO) recommends zinc supplements for 6- to 9-mo-old infants where food rich in zinc may be inaccessible (21).

In our study, serum zinc levels were found to be lower in formula-only fed infants, but the difference was not statistically significant. Onag and Taneli (22) compared serum zinc levels in 10 BMO fed infants and with those in 10 formula-only fed infants and found that BMO fed infants had levels of 78 ± 17.8 and 117 ± 23.6 μg/dL in the sixth and 12th months respectively, while formula-only fed infants had levels of 64.9 ± 20.5 and 80.9 ± 17.0 μg/dL in the same months. They reported that serum zinc levels increased significantly as the BMO fed infants grew up, while the increase in formula fed infants was not statistically significant.

Other studies reported that zinc, iron, and copper concentrations in BM are not dependent on mothers’ diet (4-7). Ergul et al. reported that as lactation progresses, the concentrations of these elements decrease in BM (7). Prasad reported in their study that BM fed infants may have low zinc levels because of low zinc levels in BM (23). Ergul et al. reported that zinc deficiency may develop in preterm infants fed with BMO, so they recommend zinc support for preterm infants rather than term infants (7). We did not consider preterm infants in our study and so we did not do a preterm-term comparison. However, we found that BMO fed infants had higher serum zinc levels: BM+formula and formula-only fed infants had lower levels of serum zinc. When the immune system and frequent infection development are considered, we think that zinc support should be recommended for formula fed infants. In our literature review, we found one study that compared serum zinc levels of 6 mo old BMO fed infants and formula fed infants. Lombeck and Fuchs compared serum zinc and copper levels of 129 term infants at birth and at the fourth month and found that formula fed infants had lower levels of zinc and copper compared to BM fed infants (24).

Copper, found in various foods, has many roles in many enzyme systems. Copper is also known to be necessary for iron absorption and hemoglobin synthesis. However, copper levels in BM are not affected by the mothers’ diet and copper supplements for mothers do not increase copper levels in BM (4, 5). Since copper accumulation in fetuses happens in the last 3 mo, preterm infants have insufficient copper storage (6). In the literature, it is reported that mothers who have preterm infants have lower levels of copper in their BM. On the other hand, term infants have enough copper storage and do not require copper supplements (7).

There are studies conducted about copper in BM; however, no study was found in the literature that compares zinc and copper levels and serum copper levels in 0–6 mo infants fed with BM and those fed with formula, except Lombeck and Fuchs. In our study, 23.5% of the formula fed infants, 8.8% of the BM+formula fed infants, and 5.3% of the BMO fed infants had low levels of copper that were significant. Based on these results, we think that copper levels in BMO fed infants are sufficient; however, formula fed infants may be given copper support or the copper content of the formulas may be increased. More studies are needed since not enough studies have been conducted about this subject.

Vitamin A is one of the necessary vitamins for the human body. Vitamin A plays important roles in vision, changes in epithelia cells, growth, reproduction and the immune system (25). In pregnant women, about 50% of the serum vitamin A level may be passed on to the fetus through the placenta. Ninety percent of vitamin A is stored in the liver; however, newborn liver storage is not adequate and it should be supplemented by BM. According to WHO criteria, serum vitamin A deficiency ratio of >5% is an indication of an important public health problem (26). Studies conducted in Turkey showed that vitamin A deficiency in children younger than five was at 30% in Ankara, in Erzurum this ratio was 10% in 6-mo- to 6-y-old children, and in Izmir, the ratio was 15.6% in 6- to 59-mo-old children (27–29).

In our study, 19.9% of the BMO fed infants, 30.8% of the BM+formula fed infants, and 47.1% of the formula fed infants had low levels of vitamin A, which was also statistically significant. Our study was among the few, if any, in making these comparisons and we feel that it contributes to the literature. We think that more studies are needed to understand whether or not formula fed infants need vitamin A supplementation after 6 mo or the vitamin A content of formulas should be increased.

As a result, we think that formula fed infants potentially have low levels of copper and vitamin A in the sixth month and may be offered supplements and/or formula mineral and vitamin contents should be enriched. BM fed infants also frequently have deficiency of these minerals and vitamins, and evaluation of BMO fed infants may be appropriate. There are not many studies that compare feeding type and serum zinc, copper, and vitamin A levels, so we think that further studies are needed.

Limitations of the study

In our study, preterm infants were not evaluated and so we did not compare trace element and vitamin A levels between term and preterm infants based on feeding
type. The number of infants who were fed with only formula was low and the types of formula used in feeding were unknown, which was another limitation. New studies are needed that include these groups.

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REFERENCES