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ARTICLE

EVALUATION OF MICRONUTRIENT DEFICIENCIES IN HEALTHY INFANTS AGED 6-12 MONTHS: IS ROUTINE SCREENING REQUIRED?

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ABSTRACT

Hidden but preventable micronutrient deficiencies in infancy threaten growth, immunity, and neurodevelopment worldwide. Early detection of these abnormalities, especially during the transition from exclusive nursing to supplemental feeding, is crucial for lifetime health. This cross-sectional study examined 500 healthy Turkish 4–12-month-olds who attended normal pediatric checkups. Retrospective blood count, ferritin, folic acid, vitamin B12, and 25(OH) vitamin D levels were examined. Anemia was found in 48.6% of babies, whereas ferritin, vitamin B12, and vitamin D deficits were found in 28.6%, 20.4%, and 33.6%. Vitamin B12 deficiency peaked at 69.3% between 6 and 9 months, when supplemental feeding began. Despite national iron and vitamin D prophylactic efforts, subclinical deficiencies persist, suggesting maternal and dietary inadequacies. These findings highlight the importance of maternal nutrition, infant supplements, and targeted public health measures for neurodevelopmental outcomes and healthy newborn growth. Strengthening preventive strategies could turn silent inadequacies into healthy generations.

Keywords: Anemia; Infant; Iron deficiency anemia; Micronutrient deficiency;Vitamin B12 deficiency; Vitamin D deficiency

АБСТРАКТ

Скрытые, но предотвратимые дефициты микроэлементов в младенчестве угрожают росту, иммунной системе и нейроразвитию по всему миру. Раннее выявление этих отклонений, особенно в период перехода от исключительно грудного вскармливания к дополнительному питанию, имеет решающее значение для здоровья на протяжении всей жизни. Это поперечное исследование охватило 500 здоровых турецких детей в возрасте от 4 до 12 месяцев, которые проходили обычные педиатрические осмотры. Были исследованы ретроспективные показатели крови, уровень ферритина, фолиевой кислоты, витамина B12 и 25(OH) витамина D. Анемия была обнаружена у 48,6% детей, тогда как дефицит ферритина, витамина B12 и витамина D был обнаружен у 28,6%, 20,4% и 33,6% соответственно. Дефицит витамина B12 достиг максимума в 69,3% в возрасте от 6 до 9 месяцев, когда началось дополнительное кормление. Несмотря на национальные профилактические меры по железу и витамину D, субклинические дефициты сохраняются, что указывает на недостатки в питании матерей и рационе. Эти результаты подчеркивают важность материнского питания, добавок для младенцев и целенаправленных мер общественного здравоохранения для нейроразвития и здорового роста новорожденных. Укрепление профилактических стратегий может превратить молчаливые недостатки в здоровые поколения.

Ключевые слова: анемия; младенец; железодефицитная анемия; дефицит микроэлементов; дефицит витамина B12; дефицит витамина D

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INTRODUCTION

Nutrients for the human body are classified as micronutrients or macronutrients. Micronutrients are substances, like vitamin s and minerals, that are needed in smaller quantities (<100 mg/dl) for the management of growth and development, according to the World Health Organization (WHO).[1] The most well-known micronutrient deficiencies are those brought on by iron, vitamin D, vitamin B12, and folic acid deficiency.[1]

Breast milk is the ideal food for a baby's healthy growth and development from birth. Its composition changes according to the baby's needs and supports the immune system. It contains water, carbohydrates (such as lactose and oligosaccharides), proteins (casein and whey), fats (including DHA and omega-3), vitamins, minerals, hormones, and live cells. Colostrum, the first milk produced, is rich in nutrients and antibodies. Transitional and mature milk change in composition over time: foremilk is more watery and hydrating, while hindmilk is fattier and more filling. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months. From six months onward, complementary foods should be introduced while continuing breastfeeding until at least two years of age. Breast milk protects babies against infections, reduces the risk of allergies, and supports brain development. For mothers, it helps the uterus return to its normal size and reduces the risk of certain cancers. Breast milk contains micronutrients, even in smaller amounts, that are bioavailable. Certain micronutrient physiological requirements are met by prenatal reserves for the first six months; however, early prophylactic administration of vitamin D and iron is recommended to prevent such deficiencies.[2]

The American Academy of Pediatrics (AAP) recommends iron prophylaxis for term infants who are exclusively breastfed from the fourth to sixth months of life, a practice that our country has been implementing since 2004, with a daily iron prophylaxis of 10 mg.[3] In addition, standard blood tests performed between the 9th and 12th months are used to assess iron deficiency anemia in infants who have received iron prophylaxis.[3]

Calcidiol (25(OH)D) is the main form of vitamin D, which is derived from both foods and endogenously produced sources, and is a vital indicator of the body's overall vitamin D status. Pigmentation of the skin, season, sunshine exposure, and nutritional vitamin D intake are some of the variables that affect normal baseline levels. Vitamin D deficiency in mothers leads to insufficient vitamin D stores in babies, with maternal 25(OH)D levels below 10 ng/mL being the most significant risk factor. [4]The worldwide consensus suggests giving 400 international units (IU) of vitamin D to all infants on the first day of life, without regard to their nutritional state. [5] The Turkish Ministry of Health suggests 400 IU of vitamin D during the first year of life to avoid rickets and vitamin D deficiencies, which is consistent with the AAP guidance.

Vitamin B12, which is required for deoxyribonucleic acid (DNA) and hemoglobin (Hb) synthesis, is primarily derived from animal sources and can cause megaloblastic anemia, increased serum homocysteine levels, and potentially neurological consequences. [6] Vitamin B12 is stored in the fetus's liver tissue during pregnancy, and children born to mothers with normal serum levels have normal concentrations. Healthy infants require 0.4 μg/day for the first six months, increasing to 0.5 μg/day in the second semester due to breast milk intake. [7] Monitoring the the mother's vitamin B12 levels is critical for fetal development, and children who have enough serum B12 levels have normal levels between 6 and 8 months of age. [8-10]

Folic acid (vitamin B9), which is required for DNA synthesis, repair, and methylation, is rarely deficient in infants due to the sufficient amounts provided by breast milk. Even in cases where maternal serum levels are below optimal, this rare condition maintains. Folic acid deficiency in mothers is a risk factor

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defects in the neural tube, so prenatal supplementation is advised. [11]

Micronutrient malnutrition is a significant public health issue affecting children, potentially leading to systemic disorders. Our country recommends hemogram screenings at nine months, but other micronutrient level evaluations may lead to unnecessary screenings. The study aims to determine the frequency of micronutrient deficiencies in Turkish infants less than one year old, focusing on iron, folic acid, vitamin B12, and vitamin D, and determine if routine screening is necessary.

MATERIAL AND METHODS

The study involved 500 infants aged 4-12 months who underwent routine check-ups at outpatient pediatric clinics of university hospital between June 2023 and June 2024. Data was retrospectively analyzed from the hospital records. This study was approved by the Ethics Committee of Bursa Yüksek İhtisas Training and Research Hospital (Decision No: 2011-KAEK-25 2022/6-13, Date: 01.06.2022). Infants with a history of term birth who were regularly monitored, had no chronic disease or continuous medication use, and were not receiving treatment for anemia due to micronutrient deficiency were included in the study. All infants included in the study were exclusively breastfed for six months and started on vitamin D supplementation at birth, and iron prophylaxis was initiated from the 4th month of age in accordance with the recommendations of the Turkish Ministry of Health and WHO. Infants_with a history of preterm birth, those whose mothers were vegetarian or followed a special diet, those with chronic or hematologic disorders, and those receiving treatment for anemia due to micronutrient deficiency were excluded from the study.

The patients' complete blood parameters (CBC), ferritin, folic acid, vitamin B12, and calcidiol (25(OH)D) tests were evaluated. CBC of all cases was performed in the Biochemistry Department Laboratory with an automatic blood counting device BC 6800 (Mindray Inc., Mahwah, NJ). Calcidiol (25(OH)D) was measured with an enzyme immunoassay developed by Abbott Inc. (Core Labs.). Serum vitamin B12 and folic acid levels were measured by the electrochemiluminescence method with the i2000 hormone analyzer developed by Abbott Inc. (Core Labs., Abbott Inc., Des Plaines, IL). Serum ferritin levels were measured using the Abbott Inc. determined by automated microparticle enzyme immunoassay method using the c16000 Architect device developed by (Core Labs., Abbott Inc., Des Plaines, IL).

Patients were compared to lower limits considered normal for their age range, based on reference values accepted by WHO. In all of our cases, the lower limit of hemoglobin (Hb) was set at 11 g/dL to define anemia.[12] The lower limit for ferritin deficiency was established at 12 ng/ml.[12] The lower value for vitamin B12 was 200 pg/ml (deficiency); the limit for borderline low (insufficiency) vitamin B12 was determined to be 200-300 pg/ml, and the lower value for folic acid was 3 ng/ml.[13,14] The lower value for 25 (OH) D was set at 20 ng/ml.[15] According to the Global Consensus Recommendations, a serum 25(OH) D concentration of less than 12 ng/mL was considered to be diagnostic for vitamin D deficiency, while concentrations between 12-20 ng/mL were considered vitamin D insufficiency, and concentrations above 20 ng/mL but less than 50 ng/mL were considered normal. [15]

Statistical analysis

Statistical analysis of the data was performed using the IBM SPSS 29.0.2.0 (IBM Corp. Released 2023). IBM SPSS Statistics for Windows, Version 29.0.2.0 (Armonk, NY: IBM Corp.) statistical package program. For descriptive findings, variables determined by counting were expressed with numbers and percentages, and variables determined by measurement were expressed with mean ± standard deviation (s.d.) or median (minimum value-maximum value), considering the

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conformity of the data to normal distribution. The distribution feature of variables determined by measurement was evaluated with the Kolmogorov-Smirnov test. For variables determined by measurement, a t-test or Mann-Whitney U test was used, considering the conformity of the data to normal distribution. Since the blinded variables did not comply with normal distribution in the correlation assessment, the Spearman correlation test was used. The significance level was accepted as $p < 0.05$.

RESULT

A total of 500 infants, with 55.4% (n=277) being male and 44.6% (n=223) being female (male/female ratio 1.2). The mean age of the patients was 8.57±2.02 months (range: 4–12 months). The mean age of females was 8.7±2.0 months and 8.5±2.1 months for males, with no significant difference (p=0.400).

Hb value of the cases was 10.9±1.40 gr/dl (min: 6.1–max: 14.6); the median hematocrit (Hct) value was 33 (min: 17.4–max: 73.5). The median Mean Corpuscular Volume (MCV) was 75.3 (min: 50.3–max: 123.5), and the median Red Cell Distribution Width (RDW) was 14.2 (min: 11.2–max: 28.1) **(Table 1).** The median ferritin value was 20 (min: 1–max: 150); the vitamin B12 value was 340 (min: 50–max: 966); the folic acid value was 18 (min: 2–max: 24); and the 25 (OH) D value was 25 (min: 5–max: 91). While Hb, MCV, and ferritin were significantly higher in females, RDW was significantly higher in males (p<0.05). **(Table 1).**

Table1. Complete blood count parameters of infants

Parameters	Female (n=223)	Male (n=277)	*p*
Hb (mean±SS)	11.2±1.3	11.0±1.5	**0.042***
Hemotokrit (median)	33.4 (17.4-40.9)	32.7 (18.8-73.5)	0.055**
MCV (median)	76.3 (50.8-123.5)	74.6(50.3-98.7)	**0.000****
RDW (median)	14 (11.2-24.5)	14.5 (11.6-28.1)	**0.000****
Ferritin (median)	24 (1-150)	18 (2-133)	**0.031****
Folic acid (median)	18 (5-24)	18 (2-24)	0.357**
Vitamin B12 (median)	332 (80-966)	345 (50-954)	0.081**
25(OH)D (median)	28 (5-80)	25(5-91)	0.346**

*t test used, Mann Whitney U test was used, MCV: mean corpuscular volume, RDW: red celldistribution width

Anemia was detected in 48.6% (n=243) of the cases. Ferritin deficiency was seen in 52.7% (n=128) of the cases with anemia, vitamin B12 deficiency was seen in 30.5% (n=74), vitamin B12 was seen as borderline low in 24.7% (n=60), and vitamin D deficiency+insufficiency was seen in 41.6% (n=101). Of the 115 cases with anemia (Hb: ≤ 11 gr/dl) and a normal ferritin value (ferritin > 12 ng/ml), 44.3% (n=51) had vitamin B12 deficiency, 24.3% had vitamin B12 insufficiency (n = 28), and 0.2% (n = 1) had folic acid deficiency. When 257 cases without anemia were investigated, 5.8% (n=15) had ferritin deficiency, 10.9% (n=15) had vitamin B12 deficiency, and 17.1% (n=44) had vitamin B12 insufficiency.

When micronutrient deficiencies independent of Hb value were examined, ferritin deficiency was found in 28.6% (n=143) of the cases, vitamin B12 deficiency was found in 20.4% (n=102), and vitamin B12 insufficiency was found in 20.8% (n=104). Folate deficiency was found in 0.4% (n=2) of the cases, and vitamin D deficiency+insufficiency and were found in 33.6% (n=168) **(Table 2.)**

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Table 2. Micronutrient Deficiencies

Parameter	Cut-off	n (%)
Ferritin deficiency (ng/mL)	<12	143 (28.6)
Vitamin B12 deficiency (pg/mL)	<200	102 (20.4)
Vitamin B12 insufficiency (pg/mL)	200–300	104 (20.8)
Folic acid deficiency (ng/mL)	<3	2 (0.4)
Vitamin D deficiency/insufficiency (ng/mL)	<20	168 (33.6)

A low level significant relationship was found between Hb and vitamin B12, folic acid, and vitamin D. A low-level significant relationship was found between vitamin D and vitamin B12, folic acid, and ferritin. A very low-level positive significant relationship was found between ferritin and folic acid and vitamin B12 **(Table 3).**

Table3. Correlation Analysis of Micronutrient Parameters

Variables	Spearman's rho	p-value
Hb – Ferritin	0.559	<0.001
Hb – Vitamin B12	0.311	<0.001
Hb – Folic acid	0.225	<0.001
Hb – 25(OH)D	0.294	<0.001
Ferritin – Vitamin B12	0.106	0.018
Ferritin – Folic acid	0.151	0.001
Ferritin – 25(OH)D	0.243	<0.001
Vitamin B12 – Folic acid	0.218	<0.001
Vitamin B12 – 25(OH)D	0.246	<0.001
Folic acid – 25(OH)D	0.205	<0.001

Ferritin deficiency peaked at 6–9 months (65%), vitamin B12 deficiency was most common at 6–9 months (69.3%), and vitamin D deficiency/insufficiency was highest at 6–9 months (64.3%).

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Table 4. Age Distribution of Micronutrient Deficiencies

Parameter	4–5 months (%)	6–9 months (%)	10–12 months (%)
Ferritin deficiency, n=143	9 (6.3)	93 (65.0)	41 (28.7)
Folic acid deficiency, n=2	0 (0.0)	2 (100.0)	0 (0.0)
Vitamin B12 deficiency, n=102	11 (10.5)	72 (69.3)	21 (20.2)
Vitamin B12 insufficiency, n=104	8 (7.8)	70 (68.6)	24 (23.6)
Vitamin D deficiency/ insufficiency, n=168	15 (8.9)	108 (64.3)	45 (26.8)

DISCUSSION

Breast milk is the best food for babies, especially in the first 6 months. It contains many important nutrients, but some, like vitamin D, vitamin B12, and iron (ferritin), may not be enough if the mother's levels are low. Folic acid is usually enough in breast milk if the mother eats well and has good levels. So, while breast milk is very nutritious, some vitamins may need to be supplemented to keep the baby healthy. We evaluate the micronutrient deficiency frequency in healthy infants, aiding early diagnosis and treatment, and suggest incorporating it into screening programs alongside iron and vitamin D prophylaxis programs. Our research revealed that B12 deficiency was significantly higher in the 6–9-month group (69.3%) compared to the 10–12-month (20.2%) and 4–5-month (10.5%) groups. Even with prophylactic programs, the rates of ferritin and vitamin D deficiencies were significantly higher in the 6–9-month period compared to the 4–5- and 10–12-month periods. Identifying micronutrient deficiencies at such high rates during this critical time for growth and neurological development is crucial. Future disorders can be avoided by assessing risk levels and putting preventative measures in place, especially when breastfeeding is discontinued and complementary food is introduced.

Around 40-50% of children in developing countries suffer from iron deficiency (ID), while ID prevalence ranges from 9-34% and iron deficiency anemia (IDA) prevalence between 3-8% in Europe. [16,17] Yalçın et al. found an anemia prevalence of 7.3% in healthy children aged 1-2. [18] The Iron-like Turkey Project significantly reduced the prevalence of IDA in Turkey, with a low incidence of anemia among children, according to previous hospital-based data (21-35%). [18] Our study revealed that anemia was more prevalent in boys than in girls; only ferritin deficiency without anemia was 5.8%, and ferritin deficiency with anemia was 25.6%. Ferritin deficiency was 6.3% during the first 4-5 months and increased to 65% at 6-9 months. Since iron prophylaxis was initiated at 4 months and the effects of the treatment were observed after 1 year of age, our study's high anemia rate may be explained by the fact that other studies were carried out between 1 and 2 years of age.

Vitamin D deficiency is a global issue with varying prevalence rates across different regions. Gordon et al. revealed 12.1% of infants aged 8-24 months had vitamin D deficiency. [19] In our country, vitamin D supplementation for infants under one year has significantly reduced rickets from 6% in 1998 to 0.1% in 2008. [4] This is in support of the 2005 vitamin D supplementation program, which provides free vitamin D to infants. According to Yeşiltepe-Mutlu et al.'s research, 7% of children under the age of one have vitamin D deficiency, while 6% have vitamin D

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insufficiency. [20] 25 (OH)D levels are higher in Turkey during the first year of life, and vitamin D deficiency and insufficiency are less common while compared with adolescents. Vitamin D deficiency rates, however, continue to exceed 10% in some regions. [20] Gül et al. found that 10.5% of children aged 3-36 months had 25(OH)D levels ranging from 12 to 19 ng/mL, while 2.1% had levels less than 12 ng/mL. [21] Vitamin D deficiency was found in 13.9% of infants under the age of one year. [22] Vitamin D in the fetus depends entirely on maternal levels, as 25(OH)D crosses the placenta during pregnancy. Maternal deficiency can lead to low neonatal vitamin D, increasing the risk of rickets and poor bone development. WHO recommends daily supplementation (400–600 IU) during pregnancy, especially for at-risk women. Ensuring adequate maternal vitamin D helps prevent deficiency in newborns and supports healthy fetal development. Our study revealed that 33.6% of infants had 25(OH)D levels below 20 ng/ml. Infants aged 6-9 months have a higher rate of vitamin D deficiency (64.3%) compared to those aged 4-5 months (8.9%) and 10-12 months (28.6%). This may be due to frequent vaccination visits and easier access to vitamin D at the first 6 months. However, there may be deficiencies in informing families about vitamin D use until the age of one, and maternal vitamin D stores may not be sufficient in the 6th month.

Vitamin B12 deficiency is a significant public health issue, with prevalence rates ranging from 40-80% in developing countries to 1-3% in developed countries. [7] The concentration of B12 in breast milk varies by region, and studies show significant variations in infant deficiency rates, influenced by dietary practices, plant-based diets, and socioeconomic status, with rates ranging from 9.8% to 63.7%. [7] In Turkey, single-center studies have reported varying rates of vitamin B12 deficiency in infants. A study conducted by Koç et al. found that 41% of infants and 72% of mothers in Şanlıurfa were Vitamin B12 deficient. [21] Yıldırmak et al found 78.2% of pregnant women and 48.9% of neonates were B12 deficient in Istanbul. [23] According to these reports, there is a significant relationship between maternal and cord blood serum vitamin B12 levels. [22,23] Developing strategies to detect vitamin B12 deficiency and ensuring adequate vitamin B12 intake during pregnancy can significantly improve the health of mothers and infants. Elgörmüş et al. found that B12 deficiency was 11.2% and insufficiency 23.5%. [24] B12 deficiency was higher in the 6–11-month group (25%) compared to the 12–23- and 24–47-month groups (5.8% and 2.8%, respectively). [24] The results suggest that low vitamin B12 levels are significant risk factors in infants, particularly from the 6th month onwards when they start consuming complementary foods. Our study revealed a 20.4% vitamin B12 deficiency rate and a 20.2% insufficiency, comparable to studies conducted in Turkey. [22-24] Vitamin B12 deficiency is most frequently found between 6 and 9 months (69.3%), and it is least common between 4 and 5 months (10.5%). Again, vitamin B12 insufficiency was found to be present at most six to nine months (68.6%). Our study's finding of increased vitamin B12 deficiency during the 6–9 month period confirms the findings of the Elgörmüş et al. study. [24] Since breastfed infants receive vitamin B12 from their mothers' milk during the first few months of life, the primary cause of these could be the mothers's vitamin B12 deficiencies. [25] Around the sixth month, infants who are exclusively breastfed have lower B12 levels. If the infant is fed a diet low in animal-derived foods during the complementary feeding period, their vitamin B12 deficiency will worsen. [25] In our study, vitamin B12 deficiency was detected less frequently in the first 5 months than in other studies conducted in Turkey. This could be because it was conducted in a maternity and child center hospital, and the mothers received better prenatal care. Our findings support the need to assess B12 deficiency in infants at risk during the complementary feeding period.

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We found a 0.4% folic acid deficiency in infants. We detected folic acid deficiency in only two cases, and they were between 6 and 9 months. Folic acid deficiency is very rare under the age of one which is consistent with previous research. [10] The reason for this may be the regular folic acid supplementation during pregnancy.

Study limitations. In addition to lacking information on the complementary feeding and growth patterns of infants, the study's retrospective, single-center design may not be representative of the general population.

CONCLUSIONS

In conclusion, in addition to vitamin D and ferritin deficiencies, vitamin B12 deficiency was also observed to be more prevalent in infants under one year of age, particularly during the 6–9 month period following the introduction of complementary feeding. These findings suggest the potential influence of maternal micronutrient status during pregnancy and lactation, as well as the adequacy and quality of infant nutrition during the complementary feeding stage. The results support the continued implementation of national iron and vitamin D prophylaxis programs. Further large-scale, multi-center studies are recommended to better understand the prevalence, risk factors, and long-term outcomes associated with early-life micronutrient deficiencies.

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DECLARATIONS

Author contribution. Study design: Elif Güler Kazancı, Data collection: Taha Metin, Ayşe Ören, Data analysis: Deniz Güven, Manuscript writing: Taha Metin, Manuscript revision: Elif Güler Kazancı, Deniz Güven Concept –E.G.K, T.M.; Design – E.G.K, T.M.; Supervision –D.G., A.Ö. ; Resources –T.M.; Materials – E.G.K, T.M ; Data Collection and/or Processing – D.G., A.Ö.; Analysis and/or Interpretation – E.G.K, T.M; Literature Search – D.G., A.Ö; Writing –. D.G.; Critical Reviews – E.G.K, T.M

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