Original Article

Serum levels of zinc, folate, and vitamin B12 in healthy children aged 3-12 months

Is routine screening necessary?

Furkan Kalayci, MD, *Metin Yigit*, MD.

ABSTRACT

الأهداف: تقييم مستويات مصل الزنك والفولات وفيتامين B12 بأثر رجعي لدى الأطفال الأصحاء الذين تتراوح أعمارهم بين 3-12 شهرًا.

المنهجية: تشمل هذه الدراسة أطفالًا أصحاء تتراوح أعمارهم بين 3-12 شهرًا والذين حضروا إلى العيادات الخارجية للأطفال في مستشفى مدينة أنقرة بيلكنت، أنقرة، تركيا، في الفترة ما بين يناير 2020 ويوليو 2022. وتم تقييم مستويات الزنك والفولات وفيتامين ب12 في الدم. بأثر رجعي.

النتائج: من بين 495 مريضاً مسجلين في دراستنا، كان 248 (50.1%) من الإناث. كان متوسط عمر المرضى 10 (المدى:7-12) شهرًا. تم الكشف عن نقص الزنك في 24 (4.8%) من المرضى، وتم العثور على نقص فيتامين B12 في 49 (9.8%) من المرضى. لم يلاحظ أي نقص حمض الفوليك في أي مريض. لم يكن هناك ارتباط كبير بين النسب المئوية للطول والوزن للمرضى ومستويات مصل الزنك والفولات وفيتامين ب 12 ($p>0.05$).

الخلاصة: في الختام، لا نوصي بإجراء فحص روتيني لمستويات الزنك والفولات وفيتامين B12 لدى الأطفال الذين تقل أعمارهم عن 12 شهرًا دون مشاكل نشطة أو أمراض مزمنة بسبب التكاليف المرتبطة بها. نقترح أن تقييم مستويات مصل الزنك والفولات وفيتامين B12 هو نهج سريري أكثر ملاءمة لدى الأطفال المعرضين لخطر نقص المغذيات الدقيقة وفي مجموعات مختارة من المرضى.

Objectives: To retrospectively assess the serum levels of zinc, folate, and vitamin B12 in healthy children aged between 3-12 months.

Methods: This study includes healthy children aged between 3-12 months who presented to the pediatric outpatient clinics of Ankara Bilkent City Hospital, Ankara, Turkey, between January 2020 and July 2022. The levels of serum zinc, folate, and vitamin B12 were evaluated retrospectively.

Results: Of the 495 patients enrolled in our study, 248 (50.1%) were female. The median age of the patients was 10 (range: 7-12) months. Zinc deficiency was detected in 24 (4.8%) patients, and vitamin B12 deficiency was found in 49 (9.8%) patients. No folate deficiency was observed in any patient. There was no significant correlation between the patients' height and weight percentiles and their serum levels of zinc, folate, and vitamin B12 ($p>0.05$ for each).

Conclusion: In conclusion, we do not recommend routine screening for zinc, folate, and vitamin B12 levels in children under 12 months of age without active issues or chronic diseases due to the associated costs. We propose that evaluating serum levels of zinc, folate, and vitamin B12 is a more appropriate clinical approach in children at risk for micronutrient deficiencies and in selected patient groups.

Keywords: zinc, folate, vitamin B12, infant, micronutrient

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From the Department of Pediatrics, Ankara Bilkent City Hospital, Ankara, Turkey.

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Address correspondence and reprint request to: Dr. Furkan Kalayci, Department of Pediatrics, Ankara Bilkent City Hospital, Ankara, Turkey. E-mail: drfurkankalayci@gmail.com
ORCID ID: https://orcid.org/0000-0002-6702-8206

Nutrients essential for human physiology are classified into 2 primary categories: micronutrients and macronutrients. Macronutrients encompass carbohydrates, proteins, and lipids, serving as the principal constituents of bodily tissues, and the primary energy sources.[1] In contrast, micronutrients, although not contributing to caloric intake, are vital in trace amounts for numerous critical physiological processes. This category includes vitamins and minerals, which play pivotal roles in regulatory and metabolic pathways.[2] Deficiencies in either micronutrients or macronutrients can precipitate systemic dysfunctions, notably affecting growth and development. This constitutes a significant public health concern across both developing and developed nations.[3]

Breast milk is instrumental in fulfilling the micronutrient requirements of infants during the initial stages of life. Despite lower concentrations of iron, zinc, and vitamin D compared to other nutrients, the bioavailability from breast milk coupled with prenatal nutrient stores suffices to meet infants' physiological needs.[4] However, the concentration of vitamins B1, B6, and B12 in breast milk may fluctuate based on maternal diet.[3] Consequently, early prophylaxis with vitamin D and iron is advocated to mitigate potential deficiencies.[5,6]

Vitamins B12 and folate are integral to homocysteine metabolism and the biosynthesis of DNA and hemoglobin. Vitamin B12 deficiency may result in megaloblastic anemia and neurological impairments, whereas folate deficiency is linked to hyperhomocysteinemia and megaloblastic anemia. Zinc plays a critical role in bone development, immune function, skin integrity, and gastrointestinal health. Its deficiency can lead to growth retardation, increased susceptibility to infections, and dermatological issues.[4,7]

During the gestational period, vitamin B12 is stored in the hepatic tissue of the fetus. Observational studies indicate that neonates of mothers with physiologically normal serum vitamin B12 levels during pregnancy exhibit correspondingly normal vitamin B12 concentrations.[8] Conversely, children born to mothers who follow vegetarian or vegan dietary regimes or those from lower socioeconomic strata are often found to have reduced levels of this vitamin. Thus, continuous monitoring of maternal vitamin B12 levels throughout pregnancy is imperative for fetal health. Furthermore, longitudinal data have confirmed that children of mothers with adequate serum B12 levels during pregnancy also display normal levels of this vitamin during the 6-8 month age interval.[9] Additionally, folic acid deficiency in pregnant women is a recognized risk factor for neural tube defects in the developing fetus. Consequently, obstetricians universally advocate for prenatal folic acid supplementation.[10] The proactive management of maternal folic acid intake is essential for preventing folate deficiency in infants, contributing significantly to neonatal health.

The Ministry of Health of the Republic of Turkey advocates for hemogram screenings at the ninth month of age but does not extend this recommendation to the evaluation of micronutrient levels. Given the often asymptomatic nature of micronutrient deficiencies, clinicians frequently employ serum micronutrient level assessments in practice to facilitate early intervention, despite the absence of overt patient symptoms. This proactive approach, however, may contribute to unnecessary screening.

The objective of our investigation was to carry out a retrospective analysis of serum zinc, folate, and vitamin B12 levels in a cohort of healthy children aged 3-12 months.

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Methods. This retrospective study encompassed healthy children aged 3-12 months who presented to the pediatric outpatient clinics of Ankara Bilkent City Hospital, Ankara, Turkey, between January 2020 and July 2022. Inclusion criteria were children who had undergone serum zinc, folate, and vitamin B12 testing for any reason, in the absence of active health complaints, or chronic diseases, and were part of routine child health surveillance. Exclusion criteria included non-Turkish nationality, active disease states, chronic disease management, prior supplementation with vitamin B12, folate, zinc, or multivitamins, and incomplete medical records. Data on patient gender, height-weight percentiles, and serum micronutrient levels were retrospectively collected and analyzed.

The research ethics committee of Ankara Bilkent City Hospital, Ankara, Turkey, granted ethical approval for the study (approval no: E2-23-3190). The research was carried out in strict adherence to the principles outlined in the Declaration of Helsinki.

Zinc levels were quantified via atomic absorption spectrophotometry, setting the deficiency threshold at <70 µg/dL.[11] Serum folate concentrations were determined using The electrochemiluminescence immunoassay, with a deficiency defined as <5 ng/mL.[12] Vitamin B12 was assessed through The enzyme-linked immunosorbent assay, with levels <200 ng/dL indicating deficiency.[13]

Statistical analysis. The Statistical Package for the Social Sciences, version 23.0 (IBM Corp., Armonk, NY, USA) Statistics for Windows was used. Categorical variables were presented as numbers and percentages (%), while continuous variables were expressed as means and standard deviations (SD). The χ^2 test was employed to analyse categorical variables between different groups. The t-test and Mann-Whitney U test were utilized to evaluate the mean differences between the 2 groups, depending on whether the data were parametric or non-parametric. A *p*-value of <0.05 was considered significant.

Results. Our investigation encompassed a cohort of 495 subjects, comprising 50.1% (n=248) females and 49.9% (n=247) males. The median age of participants was established at 10 months, with an interquartile range of 7-12 months. Distributions of weight and height percentiles stratified by gender are delineated in Table 1.

Analysis of serum zinc concentrations revealed a median level of 88.4 µg/dL (range: 79-101.7), with zinc insufficiency identified in 24 (4.8%) subjects. Median serum folate was recorded at 19 ng/mL (range: 16-22), with an absence of folate deficiency across the study population. The median value for serum vitamin B12 was determined to be 326 ng/mL (range: 248-453), with B12 deficiency observed in 49 (9.8%) participants. Concurrent deficiencies in more than one micronutrient were not detected among any of the subjects.

Statistical analyses did not reveal any significant correlations between growth percentiles and serum levels of zinc, folate, and vitamin B12 ($p > 0.05$ for each, Table 2).

Within the subset of individuals manifesting zinc deficiency, 2 exhibited a weight percentile below the 3rd percentile, and 3 were below the 3rd percentile for height. Similarly, among those with identified vitamin B12 deficiency, 2 subjects were below the 3rd percentile for weight, and 4 fell below the 3rd percentile for height.

Comparative evaluation of mean zinc levels yielded averages of 91.2±18.1 µg/dL in male subjects and 91.8±21.7 µg/dL in female subjects. Median serum vitamin B12 levels were discerned at 241 ng/mL (range: 260-453) for males and 317 ng/mL (range: 244-452) for females. Gender-based analysis regarding the prevalence of B12 (p=0.127) and zinc deficiencies (p=0.741) indicated no statistically significant disparities.

Table 1 - Height-weight percentiles by gender.

Percentile	Weight	Height
Male		
<3	12 (4.9)	23 (9.3)
3-10	30 (12.1)	14 (5.7)
10-25	48 (19.4)	35 (14.2)
25-50	55 (22.3)	74 (30.0)
50-75	57 (23.1)	58 (23.5)
75-90	31 (12.6)	34 (13.8)
90-97	14 (5.7)	9 (3.6)
Female		
<3	13 (5.2)	17 (6.9)
3-10	37 (14.9)	30 (12.1)
10-25	53 (21.4)	46 (18.5)
25-50	57 (23.0)	67 (27.0)
50-75	38 (15.3)	50 (20.2)
75-90	29 (11.7)	25 (10.1)
90-97	21 (8.5)	13 (5.2)

Values are presented as numbers and percentages (%).

Table 2 - Zinc, folate, and vitamin B12 levels according to height and weight.

Variables	Zinc	Folate	Vitamine B12
Height	0.140	0.876	0.462
Weight	0.595	0.975	0.310

Values are presented as p-values.

Discussion. Micronutrients are indispensable for a myriad of physiological functions, with their deficiencies often manifesting clinically at advanced stages, complicating early detection. The consensus among healthcare professionals on the necessity of routine laboratory screenings for micronutrient levels in pediatric follow-ups remains elusive, especially noted within guidelines across both the United States and Europe. Nonetheless, in our jurisdiction, routine assessments for zinc, folate, and vitamin B12 in infants younger than 12 months are advocated by a significant number of practitioners. This proactive approach, albeit not uniformly recommended in standard pediatric care protocols, stems from a diverse range of clinical practices and is further influenced by the escalating instances of malpractice litigation and the grave consequences of late-detected micronutrient deficiencies. Our investigation endeavors to scrutinize serum concentrations of zinc, folate, and vitamin B12 in asymptomatic infants during standard health checks, aiming to mitigate unwarranted clinical apprehensions, circumvent superfluous diagnostic testing, and eliminate the need for invasive procedures.

Zinc's pivotal role in cellular functions, growth, immune response, and gastrointestinal health is well-documented, with its deficiency linked to an array of adverse health outcomes including growth impediments, infection susceptibility, alopecia, diarrhea, diminished appetite, and dermatological issues.[2,14-16] Despite breast milk's relatively lower zinc content, which notably decreases from the first to the twelfth month of lactation, its bioavailable trace amounts are generally adequate to fulfill the nutritional requirements of exclusively breastfed infants.[4] Literature on the prevalence of zinc deficiency in pediatric populations remains limited. A meta-analysis targeting low- and middle-income nations revealed a 17% deficiency prevalence, contrasting with our findings of a 4.8% deficiency rate among the study cohort.[17] This discrepancy may be attributable to the higher breastfeeding rates and the demographic and socioeconomic factors influencing nutritional practices.

According to prevalence studies by the International Zinc Nutrition Consultative Group, the prevalence of zinc deficiency in children under 5 in Turkey is stated to be <20%.[18] The lower rate in infants under 12 months could be due to high breastfeeding rates. However, challenges such as suboptimal breastfeeding, irregular feeding patterns due to socioeconomic constraints, and educational deficits may contribute to nutritional inadequacies. The retrospective design of our study, while providing valuable insights, inherently restricts a comprehensive elucidation of these findings.

Folic acid (vitamin B9) is integral to DNA synthesis, repair, and methylation, playing a vital role in cellular division and homocysteine metabolism.[19] Despite its critical functions, folic acid deficiency in infants is relatively rare due to the adequacy of breast milk in providing necessary amounts for the infant's needs.[20] This rarity persists even when maternal serum levels are suboptimal. Our findings corroborate the literature, with no folic acid deficiencies observed within our study cohort. The paucity of research on folic acid deficiency in preschool-aged children is notable, suggesting that the widespread availability of folic acid-enriched foods across diverse socioeconomic strata may contribute to the underrepresentation of this issue within academic investigations. Additionally, obstetricians strongly emphasize the use of folic acid supplementation during prenatal care to prevent neural tube defects, and it is typically taken regularly by pregnant women. The consistent use of folic acid supplements by mothers during pregnancy may contribute to the prevention of folic acid deficiency in infants.

Vitamin B12 shares roles with folic acid in homocysteine metabolism and is crucial for DNA and hemoglobin synthesis. Predominantly sourced from animal products, B12 deficiency can manifest as megaloblastic anemia and elevated serum homocysteine levels, potentially culminating in both transient and enduring neurological effects.[21,22] The concentration of B12 in breast milk is contingent upon maternal levels, with studies highlighting significant regional variations in deficiency rates among infants.[23] For instance, investigations in In Guatemala, research findings have shown a prevalence of vitamin B12 deficiency at 35.1% among children aged 6-11 months, whereas another study has documented a deficiency rate of 63.7% in children aged 1-6 months.[19,24] Moreover, an investigation by Azad et al[25] has reported a vitamin B12 deficiency in 22% of children aged 1-12 months. Our analysis revealed a 9.8% deficiency rate, underscoring the impact of dietary practices, the prevalence of plant-based diets, and socioeconomic factors on B12 levels.

The practices surrounding laboratory and screening protocols for infants during routine check-ups exhibit considerable international variation, reflecting differing national health guidelines. For example, while the American Academy of Pediatrics endorses hemogram screenings for at-risk infants aged 9-12 months, our local guidelines recommend both hemogram and hematocrit assessments at 9 months.[26,27] Notably, screenings for micronutrients within the first year are generally discouraged in Western guidelines. The identification of micronutrient deficiencies in our cohort, absent a correlation with growth percentiles, raises questions regarding the necessity of these screenings for asymptomatic infants exhibiting normal growth patterns. This highlights a gap in the literature, advocating for a more tailored approach to micronutrient screening in pediatric populations.

Our research lends support to the recommendation against routine trace element screening in healthy, symptom-free children within our region. Decision-making regarding such screenings should be nuanced, taking into account regional health statistics, dietary norms, and risk factors to circumvent unwarranted testing in populations not deemed at risk.

Study limitations. Our investigation, characterized by its single-center and retrospective design, may not comprehensively represent the broader population. The unique socioeconomic and educational landscape of Ankara, as Turkey's capital, could skew the prevalence of micronutrient deficiencies relative to more rural settings, potentially indicating lower deficiency rates within our study cohort. Nonetheless, the substantial size of our participant group and the scarcity of analogous research in existing literature underscore the significance and contribution of our study. A notable limitation is the absence of data regarding maternal dietary habits, which, while impactful for certain micronutrients, is somewhat mitigated for zinc and folate due to their lesser dependence on maternal diet. However, this factor presents a more pronounced concern for vitamin B12, suggesting the need for future research to explore maternal B12 levels and their correlation with infantile deficiency risks.

In conclusion, micronutrient deficiencies pose a global health challenge, transcending geographical and socioeconomic boundaries. Despite the necessity for only trace amounts, the potential for overlooked deficiencies due to subtle clinical manifestations (compounded by a heightened vigilance stemming from malpractice litigation concerns) has prompted clinicians towards over-screening. Our findings highlight that routine screening for zinc, folate, and vitamin B12 in asymptomatic infants, exhibiting normal growth and devoid of clinical complaints, may lead to unwarranted healthcare expenditures and the risk of unnecessary invasive interventions. Consequently, we advocate for a selective approach to micronutrient screening, tailored to individual patient risk profiles and clinical indications, to optimize care and resource allocation effectively. This strategy aligns with a judicious use of healthcare resources, emphasizing the need for targeted interventions based on specific vulnerabilities rather than broad-based screening protocols.

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