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Age-Specific Prevalence of Vitamin B12 and Folate Deficiency in Children and Adolescents: A Six-Year Cross-Sectional Study

Authors & Affiliations

[1]Büşra Demirci, [2]Halil İbrahim Yakut

[1]Ankara Bilkent City Hospital, Department of Pediatrics, Ankara, Türkiye

Corresponding Author: Büşra Demirci, M.D., Ankara Bilkent City Hospital, Department of Pediatrics, Ankara, Türkiye
E-mail: bozuyukbusra@gmail.com

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Abstract

Background: Vitamin B12 and folate are essential micronutrients for normal growth, neurodevelopment, and hematopoiesis in children. However, age-specific data on the prevalence of vitamin B12 and folate deficiency in pediatric populations remain limited.

Methods: This cross-sectional study retrospectively analyzed serum vitamin B12 and folate measurements obtained from children and adolescents aged 0–18 years who attended a general pediatrics outpatient clinic at a tertiary care hospital between 2020 and 2025. Participants were stratified into four age groups (0–2, 2–6, 6–12, and 12–18 years). Vitamin B12 deficiency was defined as <200 pg/mL and borderline deficiency as 200–300 pg/mL, while folate deficiency was defined as <5 ng/mL. Age- and sex-specific prevalence rates were evaluated, and annual mean vitamin levels were assessed.

Results: A total of 196,091 serum measurements were analyzed over the six-year period. Vitamin B12 deficiency and borderline low vitamin B12 levels were observed across all age groups, with the highest prevalence in children aged 0–2 years and in adolescents. Folate deficiency was uncommon in early childhood but increased markedly during adolescence. Children aged 2–6 years demonstrated the lowest prevalence of both vitamin B12 and folate deficiency. No significant differences were observed between sexes. Annual mean serum vitamin B12 levels remained relatively stable, whereas serum folate levels showed notable interannual variability.

Conclusion: Vitamin B12 and folate deficiency exhibit distinct age-related patterns during childhood and adolescence. Early childhood and adolescence represent vulnerable periods for vitamin B12 insufficiency, while folate deficiency is primarily an adolescent concern. These findings highlight the importance of age-targeted nutritional surveillance and preventive strategies in pediatric care.

Keywords: Vitamin B12; Adolescents; Micronutrient deficiency

INTRODUCTION

Vitamin B12 and folic acid are essential micronutrients that play a fundamental role in normal growth, cognitive development, DNA synthesis, and cellular division, as well as in erythrocyte maturation and myelination of the nervous system (1,2). These functions render both vitamins particularly critical during childhood, a period characterized by rapid somatic growth and intense neurodevelopmental activity. Although overt clinical manifestations of deficiency may not be immediately apparent during infancy, especially in the early stages, inadequate cobalamin and folate status can exert deleterious effects on neurodevelopmental outcomes later in life (3).

Childhood and adolescence represent critical windows of heightened vulnerability to micronutrient deficiencies due to increased nutritional demands associated with accelerated physical growth and ongoing psychological and neurocognitive development (4). During these stages, insufficient intake or impaired absorption of essential vitamins may have lasting consequences. Cobalamin deficiency, in particular, is frequently associated with vegetarian or restrictive dietary patterns, as well as with inadequate maternal and childhood nutrition, a problem that remains prevalent in low- and middle-income countries (5).

Folate deficiency classically results in megaloblastic anemia and neutrophil hypersegmentation. The hematologic manifestations of vitamin B12 deficiency closely resemble those of folate deficiency and may additionally include neutropenia. Neurological involvement is a hallmark of cobalamin deficiency and varies by age. In infants, common neurological

features include irritability, hypotonia, developmental delay or regression, and involuntary movements, most notably coarse tremors. In older children, vitamin B12 deficiency may present with impaired linear growth and poor academic performance, whereas adolescents and adults may develop sensory disturbances, paresthesias, peripheral neuropathy, or psychiatric manifestations. Cutaneous findings, such as hyperpigmentation of the knuckles, palms, and soles, are also frequently reported in children and adolescents with cobalamin deficiency (6).

Assessment of vitamin status in routine clinical practice primarily relies on the measurement of serum vitamin B12 and folate concentrations. In cases where deficiency is strongly suspected, functional biomarkers such as homocysteine and methylmalonic acid (MMA) are often utilized to support the diagnosis. However, it should be noted that universally accepted, age-specific cutoff values for both serum and functional markers of cobalamin deficiency have not been well established, particularly in pediatric populations (7,8). This limitation complicates the interpretation of laboratory findings and underscores the need for population-based data.

Data from the National Health and Nutrition Examination Survey (NHANES) indicate that the prevalence of vitamin B12 deficiency ranges from 2.6% to 7.5%, depending on the survey year (9). Another NHANES analysis reported prevalence rates between 1.6% and 2.6% (10). Despite these data, large-scale, contemporary studies evaluating vitamin B12 and folate status in children are limited in our country.

Therefore, the primary aim of this study was to determine the age-specific prevalence of vitamin B12 and folate deficiencies in childhood and adolescence over a six-year period, thereby providing updated epidemiological data to inform clinical practice and public health strategies.

METHODS

Study Design and Setting: This cross-sectional study with retrospective data collection was conducted at Bilkent Children's Hospital, Ankara Bilkent City Hospital, between January 2020 and November 2025. The study protocol was approved by the local Institutional Ethics Committee, and all procedures were carried out in accordance with the principles of the Declaration of Helsinki.

Study Population: Medical records of children and adolescents aged 0–18 years who attended the General Pediatrics Outpatient Clinic and underwent serum vitamin B12 and folate testing during the study period were reviewed. Only children who were clinically healthy and had normal growth and developmental status at the time of evaluation were included. Patients with incomplete laboratory data or samples reported as hemolyzed were excluded from the analysis.

All patients under 18 years of age who had serum vitamin B12 and/or folate levels measured in the General Pediatrics Outpatient Clinic were eligible for inclusion. The total number of tests analyzed for each year from 2020 to 2025 was 11,790; 29,046; 40,223; 38,444; 39,940; and 37,648, respectively.

Laboratory Measurements: Venous blood samples were collected by experienced nursing staff using standardized procedures. For biochemical assessment, venous blood samples were collected into 5 mL serum separator tubes. Serum vitamin B12 and folate concentrations were measured using an automated analyzer (Atellica, Siemens Healthineers, Germany) following centrifugation at 4,000 rpm for 10 minutes. Only non-hemolyzed samples were included, as the hospital laboratory information system routinely flags hemolyzed specimens.

Vitamin B12 status was categorized as follows: serum vitamin B12 ≤200 pg/mL was defined as vitamin B12 deficiency, while levels between 200 and 300 pg/mL were classified as borderline (subclinical) deficiency.11 Folate deficiency was defined as a serum folate concentration <5 ng/mL.

Age Group Stratification: Participants were stratified into four predefined age groups: 0–2 years, 2–6 years, 6–12 years, and 12–18 years. The primary outcome was the age-specific and annual prevalence of vitamin B12 and folate deficiencies across these groups.

STATISTICAL ANALYSIS

Statistical analyses were performed using standard analytical methods. The normality of distribution for continuous variables was assessed using the Kolmogorov–Smirnov test. Categorical variables were expressed as numbers and percentages, while continuous variables were summarized as medians with minimum and maximum values. Descriptive analyses were conducted to determine the distribution of vitamin B12 and folate deficiency across age groups and study years.

RESULTS

Serum vitamin B12 and folate measurements obtained from patients who attended the General Pediatrics Outpatient Clinic of Ankara Bilkent City Hospital between 2020 and 2025 were retrospectively analyzed. The prevalence of vitamin B12 and folate deficiencies was evaluated across four predefined age groups (0–2 years, 2–6 years, 6–12 years, and 12–18 years) and stratified by calendar year. Age-specific distributions are presented in **Table 1.**

Across all age groups, the prevalence of folate deficiency (folate <5 ng/mL) was consistently lower than that of vitamin B12 deficiency. A progressive increase in folate deficiency rates was observed with advancing age, with the highest proportions detected in the 12–18-year age group. Similarly, both vitamin B12 deficiency (B12 <200 pg/mL) and borderline vitamin B12 levels (200–300 pg/mL) were more frequently observed among adolescents compared with younger age groups.

When analyses were performed irrespective of age, no significant differences were identified between female

Table 1. Age-Specific Annual Prevalence of Serum Vitamin B12 and Folate Deficiency in Children and Adolescents (2020–2025)

Age Group	Year	Folate <5 ng/mL, n (%)	Vitamin B12 <200 pg/mL, n (%)	Vitamin B12 200–300 pg/mL, n (%)
0–2 years	2020	2 (0.12)	156 (5.9)	611 (23.2)
	2021	11 (0.20)	732 (5.8)	1,870 (25.0)
	2022	16 (0.25)	650 (6.13)	2,849 (26.9)
	2023	4 (0.05)	547 (5.8)	2,455 (26.3)
	2024	3 (0.03)	455 (4.5)	2,357 (23.2)
	2025	10 (0.10)	379 (4.6)	1,777 (21.5)
2–6 years	2020	2 (0.13)	37 (1.4)	416 (15.6)
	2021	19 (0.40)	52 (0.8)	999 (14.8)
	2022	51 (0.86)	130 (1.35)	1,376 (14.3)
	2023	14 (0.20)	77 (0.82)	1,275 (13.7)
	2024	17 (0.20)	77 (0.81)	1,050 (11.2)
	2025	15 (0.24)	52 (0.63)	926 (11.2)
6–12 years	2020	19 (1.5)	57 (1.7)	738 (22.6)
	2021	47 (1.0)	87 (1.1)	1,883 (24.0)
	2022	102 (1.5)	210 (1.93)	2,328 (21.4)
	2023	32 (0.35)	150 (1.38)	2,244 (20.6)
	2024	49 (0.5)	118 (1.02)	2,008 (17.4)
	2025	71 (0.9)	84 (0.78)	1,791 (16.7)
12–18 years	2020	79 (4.3)	125 (3.8)	1,223 (37.5)
	2021	173 (4.0)	258 (3.6)	2,699 (38.5)
	2022	298 (5.23)	460 (5.0)	3,609 (39.4)
	2023	149 (1.92)	435 (4.9)	3,417 (38.3)
	2024	169 (2.33)	311 (3.22)	3,308 (34.3)
	2025	345 (5.3)	236 (2.8)	2,701 (31.6)

and male patients with respect to either vitamin B12 or folate deficiency rates.

Evaluation of annual mean serum vitamin B12 concentrations demonstrated relative stability over the study period. The mean B12 levels recorded from 2020 through 2025 were 398.5, 402.0, 400.3, 400.9, 426.6, and 422.38 pg/mL, respectively. In contrast, annual mean serum folate concentrations exhibited greater variability, measuring 13.04, 13.92, 12.54, 14.1, 21.76, and 12.9 ng/mL for the corresponding years.

Overall, while serum vitamin B12 levels remained largely stable throughout the study period, folate levels demonstrated notable inter-annual fluctuations.

DISCUSSION

In this large, single-center cross-sectional study spanning six years, we identified clear age-related patterns in serum vitamin B12 and folate deficiency among children and adolescents attending a general pediatrics outpatient clinic. Vitamin B12 deficiency and borderline low vitamin B12 levels were observed across all pediatric age groups but were most pronounced in early childhood (0–2 years) and increased again during adolescence, whereas folate deficiency was rare in younger children and predominantly observed in adolescents. In contrast, children aged 2–6 years demonstrated the lowest prevalence of both vitamin B12 and folate deficiency. No significant sex-based differences were detected. While annual mean serum vitamin B12 concentrations remained relatively stable over time, serum folate levels showed notable interannual variability. Collectively, these findings provide updated epidemiological insight into pediatric micronutrient status and underscore adolescence and early childhood as particularly vulnerable periods for vitamin insufficiency.

The high prevalence of vitamin B12 deficiency in infants and young children is consistent with previous reports indicating that this age group is particularly vulnerable due to dietary dependency on maternal nutrient stores (7,12). Exclusive breastfeeding, which is strongly promoted during the first months of life, represents an important protective factor for many nutrients; however, breast milk contains relatively low concentrations of vitamin B12, particularly when maternal stores are inadequate (7). Maternal vitamin B12 deficiency has been shown to be the primary determinant of infant B12 status, and infants born to mothers with low B12 levels are at substantial risk of developing deficiency, even in the absence of early clinical symptoms (12). Moreover, during the first two years of life, external dietary sources of vitamin B12 are limited, and routine supplementation is not universally implemented. Together, these factors likely explain the higher frequency of vitamin B12 deficiency observed in this age group.

In contrast, folate deficiency was rarely detected in the 0–2-year age group. This finding may be attributed to national and international public health strategies promoting folic acid supplementation before conception and during early pregnancy to prevent neural tube defects (13). Widespread maternal folic acid use has been shown

to significantly improve infant folate status, which may persist into early childhood, thereby reducing the risk of folate deficiency during infancy.

Children aged 2–6 years represented the group with the lowest prevalence of both vitamin B12 and folate deficiency. This observation may be explained by dietary transitions characteristic of this period. In our country, cow's milk consumption becomes more common after infancy, and cow's milk contains approximately five times more vitamin B12 than human breast milk (14,15). Additionally, the introduction of complementary foods and a more diverse diet increases external intake of both vitamin B12 and folate. Given the relatively structured feeding patterns and closer parental supervision typical of this age group, nutritional quality may be comparatively better, contributing to the lower deficiency rates observed.

In adolescence, however, both vitamin B12 and folate deficiencies were more frequently identified. This period is often marked by irregular eating habits, increased consumption of ultra-processed and fast foods, and reduced intake of nutrient-dense foods such as meat, dairy products, fruits, and vegetables (16). The combination of increased physiological requirements during rapid growth and suboptimal dietary patterns likely contributes to the higher prevalence of micronutrient deficiencies observed in this age group.

Notably, a substantial proportion of participants across all age groups exhibited borderline vitamin B12 levels (200–300 pg/mL) (11). Although these values may not meet conventional thresholds for overt deficiency, subclinical vitamin B12 insufficiency has been associated with adverse neurodevelopmental, cognitive, and hematologic outcomes (4,16). The high frequency of borderline vitamin B12 levels in our cohort underscores the importance of systematic screening, particularly in high-risk age groups, and highlights the need for early nutritional interventions. These findings further support recommendations for vitamin B12 supplementation not only in infants but also in mothers during pregnancy and lactation, as well as broader efforts to improve dietary quality throughout childhood and adolescence.

The primary strength of this study is the inclusion of a very large pediatric population, providing robust statistical power and enhancing the reliability of age-specific prevalence estimates. In addition, the use of recent, real-world data spanning a six-year period offers an up-to-date overview of vitamin B12 and folate status in contemporary pediatric practice.

Several limitations should also be acknowledged. First, the single-center design may limit the generalizability of the findings to broader pediatric populations. Second, potential influencing factors such as dietary intake, socioeconomic status, and maternal vitamin levels were not evaluated. Third, functional biomarkers of vitamin B12 status, including methylmalonic acid and homocysteine, were not assessed, which may have led to underestimation of subclinical deficiency. Furthermore, clinical outcomes and long-term neurodevelopmental consequences associated with vitamin deficiencies were not examined. Finally, the cross-sectional design precluded longitudinal follow-up, preventing assessment of temporal changes and long-term outcomes related to vitamin B12 and folate insufficiency.

CONCLUSION

This large, single-center cross-sectional study provides contemporary, age-specific data on vitamin B12 and folate deficiency among children and adolescents over a six-year period. Vitamin B12 deficiency was most prevalent in early childhood and adolescence, whereas folate deficiency was predominantly observed during adolescence; children aged 2–6 years exhibited the lowest rates of both deficiencies. The high frequency of borderline vitamin B12 levels across all age groups highlights the substantial burden of subclinical insufficiency, which may remain undetected in routine practice. These findings underscore the importance of age-targeted nutritional surveillance, with particular emphasis on maternal and infant vitamin B12 support, improvement of dietary quality in adolescents, and integration of vitamin B12 and folate monitoring into preventive pediatric healthcare strategies to reduce the risk of long-term adverse outcomes.

DECLARATIONS

Ethics Approval and Consent to Participate: This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Ethics Committee of Ankara Bilkent City Hospital (TABED1-25-1972). Due to the retrospective nature of the study and the use of anonymized laboratory data obtained from routine clinical practice, the requirement for informed consent was waived by the Ethics Committee.

Consent for Publication: Not applicable. This study did not include identifiable individual-level data, images, or personal information.

Availability of Data and Materials: The datasets generated and/or analyzed during the current study are not publicly available due to institutional data protection regulations but are available from the corresponding author on reasonable request and with appropriate institutional approval.

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