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Vitamin B12 deficiency and borderline levels in children aged 0–18 years: A retrospective cross-sectional study

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Abstract

Aim: This study aimed to determine the frequency of vitamin B12 deficiency in children admitted to pediatric outpatient clinics and how it varies according to age groups.

Methodology: This retrospective cross-sectional study included children aged 0–18 years who presented to pediatric outpatient clinics between June 1 and December 17, 2024, and underwent serum vitamin B12 testing. Concentrations <200 pg/mL were classified as deficient and 200–300 pg/mL as borderline. Pearson chi-square tests were used for categorical comparisons. Supplementary mean comparisons used Welch ANOVA and Holm-adjusted pairwise Welch tests calculated from rounded summary statistics.

Results: A total of 2987 patients admitted to pediatric outpatient clinics were evaluated. B12 deficiency was found in 6.5% and borderline levels in 28.8% of the children. Deficiency was observed in 6.7% of boys and 6.3% of girls; the overall association between sex and B12 category was not statistically significant (p=0.056). The most common presenting complaints were loss of appetite (28.7%), growth retardation (21.3%), and fatigue (15.9%). The mean serum vitamin B12 level was 393±159 pg/mL. When vitamin B12 deficiency was analyzed according to age groups, the highest rate of serum vitamin B12 deficiency was observed between 14–18 years of age (8.4%) and the lowest rate was observed between 0–2 years of age (4.8%). The distribution of B12 categories differed significantly across age groups (x^2=56.003, p<0.001).

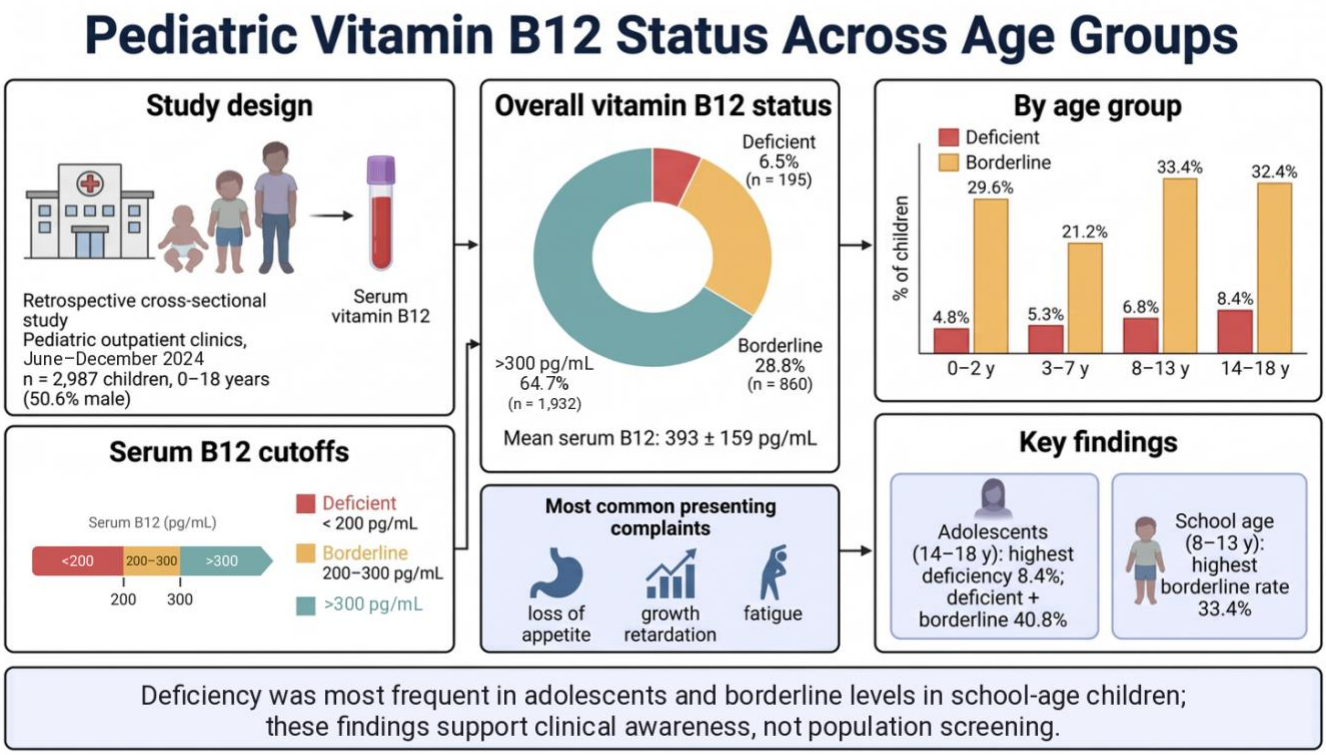
Conclusion: In this study, the rates of serum vitamin B12 deficiency in children presenting to the outpatient clinic were shown. Deficiency was found to be more frequent, especially during the adolescent period, and borderline levels were detected in 28.8% of the children. The selected sample limits generalizability; these findings alone do not establish a population screening strategy.

Keywords: Adolescent, age factors, child, cobalamin, retrospective studies, vitamin B12 deficiency

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Graphical abstract



Introduction

Vitamin B12 is an essential vitamin that cannot be synthesized by the human body and is produced primarily by microorganisms; therefore, it must be obtained exogenously, mainly through foods of animal origin (1). It functions as a cofactor in numerous metabolic pathways and plays a particularly important role in nucleic acid and protein synthesis, carbohydrate and lipid metabolism, and mitochondrial function. Inadequate vitamin B12 intake may impair cellular renewal and normal cellular function in tissues. Given its critical role in DNA synthesis, vitamin B12 deficiency can disrupt DNA synthesis and consequently lead to megaloblastic anemia (2–4).

Vitamin B12 deficiency is particularly common in low-socioeconomic-status populations and in regions where inadequate or unbalanced nutrition is prevalent. In addition to hematological manifestations, vitamin B12 deficiency may cause psychiatric and neurological disorders (3, 4). Vitamin B12 deficiency during childhood is of particular concern because it may adversely affect growth and development and potentially result in persistent neurological consequences.

Because vitamin B12 deficiency can manifest as a broad spectrum of clinical conditions, early recognition and appropriate treatment are important. This is particularly relevant during infancy, school age, and adolescence, when growth and development are accelerated. During adolescence, the increased growth rate and nutritional demands, combined with inadequate or unbalanced dietary habits, may be an important risk factor for vitamin B12 deficiency. Furthermore, vitamin B12 deficiency in children does not always present with overt hematological abnormalities; therefore, laboratory evaluation is important in clinically suspected cases (5–7).

The potential of vitamin B12 deficiency to cause neurological morbidity underscores the importance of determining its prevalence and identifying associated risk factors, particularly in developing populations (8, 9). Identifying age groups particularly vulnerable during critical periods of growth and development may facilitate early detection of at-risk children and enable timely implementation of appropriate nutritional interventions and treatment strategies. In addition, assessing population dietary characteristics and planning preventive healthcare services targeting high-risk groups may help prevent the long-term adverse consequences of vitamin B12 deficiency (4, 10).

This study aimed to determine the frequency of vitamin B12 deficiency among children presenting to pediatric outpatient clinics and to evaluate variations in serum vitamin B12 levels across different age groups. We also aimed to identify the age periods in which

deficiency was most prominent and to determine age groups at increased risk that may benefit from early detection and intervention. Although previous studies from Türkiye have reported vitamin B12 status in children, most have focused on specific age periods or selected populations, and data covering the entire pediatric age spectrum from southeastern Türkiye, a region with distinct socioeconomic and dietary characteristics, remain limited. Therefore, the present study provides region-specific data on both deficient and borderline vitamin B12 levels across all pediatric age groups in a large sample of children.

Materials and Methods

This retrospective cross-sectional study was approved by the Scientific Research Ethics Committee of the Ministry of Health, Batman Training and Research Hospital (December 18, 2024; decision number: 396). All procedures were conducted in accordance with applicable ethical requirements and the principles of the Declaration of Helsinki 2024.

Children aged 0–18 years who presented to the pediatric outpatient clinics of Batman Training and Research Hospital between June 1 and December 17, 2024, and underwent serum vitamin B12 testing were included. Patients who had received treatment for vitamin B12 deficiency were excluded. No additional exclusion criteria were applied on the basis of chronic disease, use of medications potentially affecting vitamin B12 concentrations, or vitamin supplementation. These factors were not systematically assessed.

Patient characteristics, presenting complaints, and laboratory results were retrieved retrospectively from the hospital information system and recorded using a standardized data collection form. Patients were classified into four age groups: 0–2, 3–7, 8–13, and 14–18 years.

Serum vitamin B12 concentrations were measured by electrochemiluminescence immunoassay (ECLIA) using a Roche cobas 8000 analyzer (Roche Diagnostics, Basel, Switzerland). The laboratory reference interval for serum vitamin B12 was 200–900 pg/mL. For the study analyses, concentrations were categorized as deficient (<200 pg/mL), borderline (200–300 pg/mL), or >300 pg/mL, according to the selected classification criteria (5). These analytical categories were distinguished from the laboratory reference interval; the >300 pg/mL category had no upper cutoff.

Statistical analysis

The primary analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). Supplementary analyses were conducted using Python (SciPy version 1.17.0). Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequencies and percentages. In the primary analyses, the normality of continuous variables was assessed using the Shapiro–Wilk test.

Overall associations between sex or age group and vitamin B12 category were evaluated using Pearson chi-square tests on 2×3 and 4×3 contingency tables, respectively. Exploratory category-specific comparisons by sex were performed using two-sided Pearson chi-square tests without Yates correction, comparing each vitamin B12 category with the other two categories combined in a 2×2 table. Bonferroni correction was applied across these three comparisons; adjusted p-values were calculated as min(3 × unadjusted p, 1).

A supplementary comparison of mean vitamin B12 concentrations across age groups was performed using Welch one-way analysis of variance, calculated from the reported group sizes, means, and standard deviations. Post-hoc comparisons used two-sided pairwise Welch t-tests, with Holm correction across all six age-group comparisons. These methods do not require equal population variances. Because these supplementary analyses were based on rounded summary statistics, distributional assumptions and outliers could not be reassessed, and rank-based analyses could not be performed.

Statistical significance was defined as $p<0.05$ for overall tests and multiplicity-adjusted p-values for exploratory and post-hoc comparisons.

Results

The study included 2987 pediatric patients. Demographic data and presenting complaints of the children aged 0–18 years who presented to pediatric outpatient clinics and underwent serum vitamin B12 testing are shown in Table 1. According to sex, 50.6% of patients were male. By presenting complaints, loss of appetite was the most common symptom, present in 28.7% of patients. Growth retardation was reported in 21.3%, fatigue in 15.9%, forgetfulness in 9.2%, headache and dizziness in 7.8%, tremor in hands in 4.6%, weight loss in 3.3%, nail disorders in 2.5%, hair loss in 2.3%, mouth ulcers in 1.8%, and other causes in 2.6%.

Table 2 presents the distribution of serum vitamin B12 categories by sex. The overall association between sex and vitamin B12 category was not statistically significant (Pearson $x^2=5.770$, df=2, p=0.056). Deficient concentrations were observed in 6.7% of boys and 6.3% of girls, borderline concentrations in 30.6% and 26.9%, and concentrations >300 pg/mL in 62.6% and 66.8%, respectively. Exploratory category-specific comparisons yielded unadjusted p-values of 0.626, 0.025, and 0.018, respectively. None were statistically significant after Bonferroni correction for three comparisons (adjusted p=1.000, 0.076, and 0.053, respectively).

Table 1. Demographic and clinical characteristics of the patients.

Variables		n (%)
Sex	Male	1512 (50.6)
	Female	1475 (49.4)
Presenting complaints	Loss of appetite	857 (28.7)
	Growth retardation	635 (21.3)
	Fatigue	476 (15.9)
	Forgetfulness	275 (9.2)
	Headache/dizziness	234 (7.8)
	Tremor in hands	137 (4.6)
	Weight loss	98 (3.3)
	Nail disorders	74 (2.5)
	Hair loss	68 (2.3)
	Mouth ulcer	55 (1.8)
	Others	78 (2.6)

Values are n (%); percentages use the total study population (n=2987). This table is descriptive; no inferential statistical test was applied.

Table 2. Distribution of serum B12 levels according to sex.

B12 levels (pg/mL)	Male, n (%)	Female, n (%)	p
<200	102 (6.7)	93 (6.3)	0.626
200-300	463 (30.6)	397 (26.9)	0.025
>300	947 (62.6)	985 (66.8)	0.018

Values are n (% within sex). Overall association between sex and B12 category: Pearson chi-square test (2×3), x^2=5.770, df=2, p=0.056. Category-specific p-values are from two-sided Pearson chi-square tests without Yates correction, comparing each B12 category with the other two combined (2×2); these unadjusted comparisons are exploratory. Bonferroni-adjusted p-values for <200, 200–300, and >300 pg/mL are 1.000, 0.076, and 0.053, respectively; none meet the family-wise significance level of 0.05 (unadjusted threshold 0.05/3=0.0167).

The overall mean serum vitamin B12 concentration was 393 ± 159 pg/mL. Age-specific means were 401 ± 166, 414 ± 157, 381 ± 154, and 377 ± 159 pg/mL for the 0–2, 3–7, 8–13, and 14–18 year groups, respectively. In the supplementary analysis based on rounded summary statistics, Welch ANOVA indicated a difference in mean concentrations across age groups (F[3, 1349.56]≈10.39, p<0.001). Post-hoc Welch comparisons with Holm correction showed higher mean B12 concentrations in children aged 3–7 years than in those aged 8–13 years (mean difference 33 pg/mL, adjusted p<0.001) and 14–18 years (mean difference 37 pg/mL, adjusted p<0.001). The other four comparisons were not statistically significant after correction. Vitamin B12 deficiency was observed in 6.5% (n=195) and borderline levels in 28.8% (n=860) of children. The distribution of B12 categories differed across age groups (Pearson x^2=56.003, df=6, p<0.001). Deficiency was observed in 4.8% of children aged 0–2 years and 8.4% of those aged 14–18 years; borderline levels occurred in 33.4% and 32.4% of children aged 8–13 and 14–18 years, respectively (Table 3).

Table 3. Distribution of vitamin B12 levels in pediatric age groups.

	B12 levels (pg/mL)			
Age	(Mean±SD)	<200 n (%)	200–300 n (%)	>300 n (%)
0-2	401±166	19 (4.8)	118 (29.6)	261 (65.6)
3-7	414±157	49 (5.3)	197 (21.2)	685 (73.6)
8-13	381±154	53 (6.8)	259 (33.4)	463 (59.7)
14-18	377±159	74 (8.4)	286 (32.4)	523 (59.2)
Total	393±159	195 (6.5)	860 (28.8)	1932 (64.7)

Values are mean ± SD or n (% within age group); group totals are 398, 931, 775, and 883. B12 category distribution: Pearson chi-square test (4×3), x^2=56.003, df=6, p<0.001. Supplementary comparison of means from rounded summary statistics: Welch ANOVA, F(3, 1349.56)≈10.39, p<0.001. Post-hoc two-sided Welch tests with Holm correction across six comparisons: 0–2 vs. 3–7 years, p=0.369; 0–2 vs. 8–13, p=0.137; 0–2 vs. 14–18, p=0.062; 3–7 vs. 8–13, p<0.001; 3–7 vs. 14–18, p<0.001; 8–13 vs. 14–18, p=0.603.

Discussion

According to the findings of our study, vitamin B12 deficiency was most frequently observed among children aged 14–18 years, corresponding to the adolescence period. However, when children with borderline vitamin B12 levels requiring close follow-up were also considered, vitamin B12 status among school-aged children aged 8–13 years was also found to be generally suboptimal. Childhood and adolescence are critical periods characterized by rapid growth and increased nutritional requirements. Accordingly, vitamin B12 deficiency represents an important health concern during the adolescent and preadolescent periods, which are among the most critical stages of growth and development (11).

Vitamin B12 is an essential vitamin involved in DNA synthesis, erythropoiesis, myelin formation, and neurological development. Therefore, inadequate vitamin B12 levels during childhood may have hematological, neurological, and developmental consequences. Although severe vitamin B12 deficiency is typically associated with megaloblastic anemia and neurological manifestations, mild or borderline deficiency may remain clinically silent or present with nonspecific symptoms. Therefore, early recognition of inadequate vitamin B12 status is important, particularly in children presenting with nonspecific complaints, such as fatigue, poor appetite, growth retardation, headache, dizziness, or cognitive difficulties (12, 13). In our study, poor appetite was the most common presenting complaint, reported in 28.7% of children, followed by growth retardation (21.3%), fatigue (15.9%), and forgetfulness (9.2%). These symptoms are not specific to vitamin B12 deficiency and may be associated with a wide range of pediatric conditions. Nevertheless, their relatively high frequency among children in whom vitamin B12 levels were assessed highlights the importance of considering nutritional

deficiencies in the differential diagnosis of nonspecific pediatric complaints.

Studies conducted in Türkiye and other countries have reported vitamin B12 deficiency rates ranging from 1.4% to 22% among children (14–18). The differences among these studies may be attributable to variations in age distribution, dietary patterns, socioeconomic status, geographic region, study design, sample size, and cutoff values used to define vitamin B12 deficiency. In a study conducted by Çağ et al. among children younger than 17 years, the mean vitamin B12 level was 336.6 ± 161.5 pg/mL, whereas a study conducted in Konya among individuals aged 0–24 years reported a mean level of 263 ± 127 pg/mL (14, 19). In our study, the mean serum vitamin B12 level was 393 ± 159 pg/mL, which was higher than the means reported in these studies; this difference may reflect variations in age range, study population, and assay methods.

Previous studies have frequently reported a particularly high prevalence of serum vitamin B12 deficiency among newborns, with maternal vitamin B12 deficiency considered a major contributing factor (20–23). In a study conducted by Yetim et al. among adolescents, approximately one-third of the participants were found to have either deficient or borderline vitamin B12 levels (24). In contrast to the neonatal findings, our study identified vitamin B12 deficiency in 4.8% of children aged 0–2 years. The frequency increased to 8.4% among adolescents aged 14–18 years. When children with borderline vitamin B12 levels were also included, the proportion in this age group increased to 40.8%, which is consistent with the findings of Yetim et al.

Our findings can be compared with those of recent studies from Türkiye. In a study of 3163 children from Türkiye, Sürücü Kara et al. reported vitamin B12 deficiency in 5.5% and borderline levels in 23.6% of children, with the lowest vitamin B12 levels observed in those aged 14–18 years (25). In a recent study of 234 children aged 6–18 years from Istanbul, Culha Hosceylan et al. reported a considerably higher overall deficiency frequency (40.6%), but similarly observed that deficiency was more frequent in adolescents aged 12–18 years (26). The relatively low deficiency rate in the 0–2-year age group in our study (4.8%) may be explained by several factors. First, this group included both infants and toddlers, many of whom may have already started complementary feeding with animal-source foods, which may have diluted the higher risk associated with exclusive breastfeeding in early infancy. Second, vitamin B12 supplementation during pregnancy, lactation, or infancy could not be assessed because of the retrospective design. Third, vitamin B12 levels are physiologically higher in early childhood than in adolescence, and the application of uniform cutoff values across all age groups may have led to an underestimation of deficiency in younger children (19). Therefore, the true frequency of vitamin B12 deficiency in this age group may be higher than that observed in our study.

Adolescents may be particularly vulnerable to vitamin B12 deficiency because of irregular eating patterns, meal skipping, reduced consumption of nutrient-dense foods, and increased consumption of processed and convenience foods. In addition, restrictive dietary practices, including vegetarian and vegan diets, may further increase the risk of vitamin B12 deficiency because naturally occurring vitamin B12 is predominantly found in foods of animal origin. Therefore, the high proportion of adolescents with low or borderline vitamin B12 levels in our study may partly reflect age-related changes in dietary behavior and increased nutritional requirements associated with rapid growth.

The high frequency of borderline vitamin B12 levels in school-aged children is also noteworthy. In our study, 33.4% of children aged 8–13 years had serum vitamin B12 levels between 200 and 300 pg/mL. Although borderline serum vitamin B12 levels do not necessarily indicate a clinically significant tissue deficiency, they may represent an early indicator of inadequate vitamin B12 status in some children. Therefore, the assessment of children with borderline vitamin B12 levels should incorporate dietary history, clinical findings, hematological parameters, and, when clinically indicated, additional biochemical markers. This approach may be particularly important for children with persistent symptoms or risk factors for vitamin B12 deficiency.

No statistically significant overall association was demonstrated between sex and vitamin B12 category ($p=0.056$). Although the observed proportions of borderline concentrations and concentrations >300 pg/mL differed numerically between boys and girls, none of the exploratory category-specific comparisons remained statistically significant after Bonferroni correction. These findings therefore do not provide sufficient evidence of a sex-related difference in vitamin B12 status in this sample.

Study limitations

This study has several limitations. First, its single-center retrospective design may limit the generalizability of the findings. Second, only children who underwent serum vitamin B12 testing were included, which may have introduced selection bias; therefore, the observed frequencies should not be interpreted as prevalence estimates for the general pediatric population. Third, homocysteine and methylmalonic acid concentrations were unavailable, limiting the assessment of functional vitamin B12 deficiency. Fourth, dietary habits, socioeconomic status, hematological parameters, and maternal vitamin B12 status were not evaluated, limiting the interpretation of factors potentially associated with vitamin B12 status. Potential confounding by chronic diseases, medications affecting vitamin B12 concentrations, and vitamin supplementation could not be ruled out. Finally, the supplementary comparisons of

mean vitamin B12 concentrations across age groups were calculated from rounded summary statistics rather than individual-level measurements. Consequently, distributional assumptions and outliers could not be reassessed, and rank-based analyses could not be performed. These supplementary comparisons should therefore be interpreted cautiously. The findings do not establish causal relationships or demonstrate the effectiveness of population screening or routine supplementation.

Conclusion

Among children undergoing vitamin B12 testing in pediatric outpatient clinics, deficiency was observed in 6.5% and borderline concentrations in 28.8%. The highest observed deficiency proportion occurred in adolescents aged 14–18 years, whereas the highest borderline proportion occurred at ages 8–13 years. B12 category distributions differed across age groups; supplementary mean comparisons also indicated age-related variation. No statistically significant overall association with sex was demonstrated. These findings may inform clinical awareness in symptomatic children, but the selected sample and methodological limitations preclude recommendations for population screening or routine supplementation based on this study alone.

Disclosures

Ethical Approval: Ethics committee approval was received for this study from the Scientific Research Ethics Committee of the Ministry of Health, Batman Training and Research Hospital, in accordance with the World Medical Association Declaration of Helsinki 2024, with the approval number: 396 (date: December 18, 2024).

Informed Consent: Due to the retrospective design of the study, the requirement for informed consent was waived by the ethics committee.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept — M.S., Ö.O.; Design — M.S., Ö.O.; Supervision — M.S.; Materials — M.S., Ö.O.; Data collection or processing — M.S.; Analysis and/or interpretation — M.S., Ö.O.; Literature search — Ö.O.; Writing — M.S., Ö.O.; Critical review — M.S., Ö.O.

Declaration of Generative AI and AI-Assisted Technologies: During the preparation of this work, FigureLabs was used to create the graphical abstract, and the AI figure-generation feature in BioRender was used for subsequent text edits to the figure. The author(s) then reviewed, edited, and verified all visual and scientific content for accuracy and take(s) full responsibility for the final published material.

Conflict of Interest: The authors declare that there is no conflict of interest.

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