ISTANBUL UNIVERSITY PRESS

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.1031447
İst Tıp Fak Derg 2022 / J Ist Faculty Med 2022

# THE IMPACT OF AGE, SEX, AND SOCIO-ECONOMIC STATUS ON SERUM VITAMIN B12, FOLATE CONCENTRATION AND IRON STATUS IN TURKISH CHILDREN: A CROSS-SECTIONAL STUDY

## TÜRK ÇOCUKLARINDA YAŞ, CİNSİYET VE SOSYO-EKONOMİK DURUMUNUN SERUM DEMİR, VİTAMİN B-12 VE FOLİK ASİT KONSANTRASYONLARI ÜZERİNDEKİ ETKİLERİ: KESİTSEL BİR ÇALIŞMA

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**Cite this article as:** Eroglu H, Kaytaz M, Akkaya E, Ademoglu E, Genc S. The impact of age, sex, and socio-economic status on serum vitamin B12, folate concentration and iron status in Turkish children: a cross-sectional study. J Ist Faculty Med 2022;85(4):572-80. doi: 10.26650/IUITFD.1031447

### ABSTRACT

**Objective:** The aim of this study is to evaluate the effects of age, sex, and socio-economic status (SES) on concentrations of vitamin B12 (vit-B12), folate, iron, and ferritin through the pediatric age span, and to show the relationship of these nutrients with blood count parameters such as hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and red cell distribution (RDW).

**Material and Method:** The study comprised of 30,504 venous blood samples (54.5% of girls and 45.5% of boys). The study group was stratified; group I: 1-6 years (n=3,870), group II: 7-12 years (n=11,019), and group III: 13-18 years (n=15,615). Serum vit-B12, folate, ferritin, iron and total iron-binding capacity (TIBC) were measured using a Beckman Coulter DXI 800, and the blood count was analyzed using a Sysmex XE 2100 analyzer.

**Results:** The Hb, Hct, MCV, and RDW levels were significantly different between the age groups of boys and girls (p<0.001, for all). Hb and Hct were the highest in boys aged 13-18 years, and higher than those of girls in the same age group (p<0.001). The MCV and RDW were also significantly different across the age groups (p<0.001). The iron deficiency prevalences were 12.3% and 4.2% for the girls and boys respectively. Serum folate and vit-B12 showed decrement with age across the age groups. The prevalences of vit-B12 deficiency were 27.1% and 28.3% and 6.0% and 6.8% for folate deficiency for girls and boys. Iron, TIBC,

### ÖZET

**Amaç:** Bu çalışmanın amacı, pediyatrik yaş gruplarında; yaş, cinsiyet ve sosyo-ekonomik durumun (SED) B12 vitamini, folik asit, demir ve ferritin konsantrasyonlarına etkisi, bu parametrelerin hemoglobin (Hb), hematokrit (Hct), ortalama eritrosit hacmi (MCV) , RDW gibi kan sayımı parametreleri arasındaki ilişkisinin saptanmasıdır.

**Gereç ve Yöntem:** Çalışma 30504 venöz kan örneğinden oluştu (%54,5 kız, %45,5 erkek). Çalışma grupları; grup I: 1-6 yaş (n=3.870), grup II: 7-12 yaş (n=11.019), grup III: 13-18 yaş (n=15.615). Serum B12 vitamini, folik asit, ferritin, demir, demir bağlama kapasitesi (TDBK), Beckman Coulter'ın DXI 800 analizörü kullanılarak, tam kan sayımı Sysmex XE 2100 hematoloji cihazında çalışıldı.

**Bulgular:** Kız ve erkek çocuklarının yaş grupları arasında Hb, Hct, MCV, RDW düzeyleri anlamlı olarak farklı bulundu (tüm parametreler için, p<0.001). Hb ve Hct düzeyleri 13-18 yaş grubu erkek çocuklarında en yüksek seviyede olup, aynı yaş grubundaki kız çocuklarına göre anlamlı olarak daha yüksekti (p<0.001). Demir eksikliği prevalansı, kız çocuklarda %12,3, erkek çocuklarda %4,2 idi. Serum folik asit ve B12 vitamin konsantrasyonlarında yaş grupları içerisinde yaşla azalma görüldü (sırasıyla r=-0,480, p<0,001; r=-0,377, p<0,001). Kız ve erkek çocuklarında, B12 vitamin eksikliği prevalansı sırasıyla %27,1 ve %28,3 iken, folik asit eksikliği prevalansı %6,0 ve %6,8 olarak bulundu. Demir, TDBK

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**Submitted/Başvuru:** 02.12.2021 • **Revision Requested/Revizyon Talebi:** 13.12.2021 • **Last Revision Received/Son Revizyon:** 17.06.2022 • **Accepted/Kabul:** 29.06.2022 • **Published Online/Online Yayın:** 19.08.2022

and ferritin levels were significantly different between the high and medium SES.

**Conclusion:** The results of this study are important in that the monitorization of ferritin, vit-B12, and folate levels greatly contribute to clinical practice because of the roles of vit-B12, folate, and iron in mental, emotional, and metabolic development. However, there is a need for larger and multicenter studies that can represent the nutrients of Turkish children and adolescents nationwide.

**Keywords:** Serum vitamin B-12, folic acid, iron status, iron deficiency prevalence, B-12 and folate deficiency prevalence

ve ferritin düzeylerinde yüksek ve orta SED arasında anlamlı farklılıklar görüldü (p<0,001, tüm parametreler için).

**Sonuç:** Bu çalışmanın sonuçları, ferritin, B12 vitamini, folik asit düzeylerinin mental, emosyonal ve metabolik gelişimlerindeki rolleri nedeniyle çocukluk çağı ve ergenlikte izlenmesinin klinik uygulamaya katkıları açısından önem taşımaktadır. Ancak, bu nutrientleri ülke genelinde Türk çocuk ve ergenlerindeki temsil edebilecek daha geniş ve çok merkezli çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** Serum B12 vitamini, folik asit, demir eksikliği prevalansı, B12 vitamin eksikliği prevalansı, folik asit eksikliği prevalansı

## INTRODUCTION

Adequate levels of micronutrients are essential for any stage of life, especially in childhood and adolescence. Folate and vitamin B12 (vit-B12) are crucial micronutrients for growth and development. They have indisputable roles in DNA synthesis, optimal hematopoiesis, and neuronal function. Studies have shown the association between inadequate levels of these vitamins with developmental problems and neurologic deficits (1, 2).

Micronutrient deficiency, especially iron, vit-B12, and folate, are the main causes of anemia. Vit-B12 participates in the methylation reaction of homocysteine (Hcy) and the conversion of methylmalonyl-CoA to succinyl-CoA. With inadequate levels of Vit-B12, the methylation reaction and the conversion of methyl-tetrafolate to its active form (tetrahydrofolate) are disrupted, resulting in functional folate insufficiency and macrocytic anemia. The intracellular and circulating levels of Hcy, which is an independent risk factor for atherosclerosis, are increased with a lack of vit-B12 and folate, and several potentially harmful effects of hyperHcy on the vascular endothelium and bone metabolism have been demonstrated (3, 4).

Vitamin B12 deficiency is common in developed countries, especially in vegetarians, alcoholics, and the geriatric population (1). Although some researchers have demonstrated the relationship between low folate and Vit-B12 levels and some chronic diseases in childhood, studies about the effects of low vit-B12 and folate on general health status in children and adolescents, and reference values are not certain, unlike in adults (5). National surveys investigating the prevalence of vit-B12 and folate deficiency in childhood and adolescence have shown different results in different parts of the world (6, 7). Thus, it should be noted that cultural and nutritional attitudes of populations in different parts of the world are influential on vit-B12 and folate concentrations.

Iron is an essential micronutritient for almost all living organisms with participating oxygen transport, deoxyribonucleic acid (DNA) synthesis, electron transport, and hematopoiesis, and is the most frequently seen nutritional deficiency in the world according to the World Health Organization (WHO), and its effects on the central nervous system, such as the metabolic function of the brain and roles in myelin synthesis, enhance its importance in general health (8). Cognitive, social, and emotional disturbances have been reported in adolescence due to iron deficiency (9). According to the recommendations of American Academy of Pediatrics, anemia screening in children is recommended to be performed with hemoglobin (Hb) measurement at the age of 1 year, because of the low sensitivity and specificity of Hb, the WHO recommends to use ferritin with Hb in the determination of iron deficiency (10).

In this cross-sectional study, our purpose was to evaluate the effects of age, sex, and socioeconomic status on concentrations of vit-B12, folate, iron, and ferritin through the pediatric age span, and to show the relationship of these nutrients with blood count parameters such as Hb, hematocrit (Hct), mean corpuscular volume (MCV), and red cell distribution (RDW).

## MATERIALS AND METHOD

### Study population

Thirty thousand five hundred and ninety-four venous blood samples from residual material of the Istanbul Public Health Laboratory workflow comprised our study group (54.5% of girls and 45.5% of boys; mean age: 12.1±4.3 years). The study group was stratified according to age groups; group I: 1-6 years (n=3,870), group II: 7-12 years (n=11,019), and group III: 13-18 years (n=15,615). Blood samples were collected into serum separator tubes (SST, Becton Dickinson, Plymouth, UK), centrifuged for 10 minutes at 2000 g and the supernatants were used for serum vit-B12, folate, ferritin, iron and total iron-binding capacity (TIBC) measurements. For the complete blood count (CBC), venous blood samples collected into K2–EDTA anti-coagulated tubes (Becton Dickinson, Plymouth, UK) were used, and Hb, red blood cells (RBC), MCV, RDW levels were measured. All the parameters were studied on the same day within three hours. Fasting was not required for these analyses because the majority of our study group was children.

Our study included healthy children and adolescents between 1 year to 18 years of age. Children with a history of acute or chronic illnesses or type 1 diabetes, celiac disease, renal or liver disease and those using any medications were excluded.

The current thresholds recommended by the WHO were used for serum ferritin, folate, and vit-B12, which are lower than 12 ng/mL, 4 ng/mL, and 148 pg/mL, respectively (10). Socioeconomic status was defined according to the classification of the Human Developmental Index, which includes the seven main indices (Governance, Social Inclusion, Economic Status, Education, Health, Social Life, Municipal Environmental Performance and Transportation) in 39 districts of Istanbul (11). The study group was stratified in three groups according to SES classification; group A; high human development, group B; medium development group C; low development group.

The study protocol was approved by the Ethics Committees of the Istanbul University, Istanbul Faculty of Medicine (Date: 25.2.2020, No: 332). Informed consent was received from the families of the participants.

### Methodology

The CBC was analyzed using a Sysmex XE 2100 analyzer (Sysmex Corp. Kobe, Japan). Serum ferritin, folate, and vit-B12 levels were measured using a Beckman Coulter DXI 800 (Beckman Coulter, USA) with a sandwich immunoenzymatic method, and serum iron and TIBC were determined using an AU5800 analyzer with colorimetric method. The precision results for vit-B12, folate, and ferritin were 2.3%, 5.0%, and 4.5%, respectively. The coefficient of variation (CVs) values of the hematologic parameters were lower than 2.0%.

### Statistical analysis

The data were analyzed using the SPSS 21 software (SPSS, Chicago, IL, USA). The results are expressed as mean ± standard deviation (SD). The normality of data distribution was evaluated using the Kolmogorov-Smirnov test. The Chi-square ($\chi^2$), Kruskal–Wallis, and a post-hoc analysis using the Mann–Whitney U-test was used for the variables distribution that was not normally distributed, and a student-t test was used for the variables distribution that was normally distributed . Correlation analyses were performed using the Spearman test. Binary logistic regression was used to evaluate the variables affecting the risk of iron, vit-B12, and folate deficiencies. Statistical significance was defined as p<0.05.

## RESULTS

Table 1 presents the descriptive characteristics of the study group. When the CBC parameters were compared in all age groups of the girls and boys, the Hb, Hct, MCV, and RDW levels were significantly different between the age groups; the Hb and Hct concentrations were the highest in boys aged 13-18 years (p<0.001). The Hb and Hct levels of boys aged 13-18 years were also significantly higher than those of girls in the same age group (14.5±1.2 vs. 12.6±1.2 g/dL and 43.0±3.1 vs. 38.4±2.9%, p<0.001 for both). The results of other CBC parameters, MCV, and RDW in 1-6 years, 7-12 years and 13-18 years age groups were significantly different in both sexes across the age groups of both sexes (p<0.001 for all).

The prevalence of iron deficiency was 12.3% and 4.2% for the girls and boys, respectively. When we investigated through the age groups, the prevalence was 17.3% and 4.6% for the 13-18 years' age group, 4.0% and 1.5% for the 7-12 years' age group, and 4.9% and 7.3% for the 1-6 years' age group, respectively. The prevalence of iron deficiency anemia in the 7-12 years' and 13-18 years' age groups were found significantly different between the boys and girls (p=0.009, p<0.001, respectively).

When the serum vit-B12, folate, iron, and ferritin levels were evaluated, the serum B12, folate, iron, and ferritin concentrations were significantly different through the age groups of girls and boys (p<0.001, for all). Serum iron, TIBC, and ferritin levels were also different between girls and boys in the 7-12 years' age group (p=0.033, p=0.011, p<0.001, respectively), and in the 13-18 years' age group (p<0.001, for all). However, the serum folate and vit-B12 concentrations showed a decrement across the age groups. Vit-B12 and folate levels were significantly lower in boys and girls aged 13-18 years compared with the other age groups (p<0.001, in all age groups)

The prevalence of serum vit-B12 deficiency in all study groups was 27.1% and 28.3% for the girls and boys. When we investigated through the age groups, the prevalence of B12 deficiency was 36.1% and 43.1% for the 13-18 years'; 15.3% and 16.5% for the 7-12 years' age and 7.2% and 9.7% for the 1-6 years' age group for girls and boys respectively, and the differences were significant between the age groups (p<0.001, for all). The prevalence of serum folate deficiency in all study groups was 6.0% and 6.8% for the girls and boys, respectively. Across the age groups; the prevalence of folate deficiency was 8.7% and 12.7% for the 13-18 years' age group, 0.7% and 1.3% for the 7-12 years' age group, and 0.9% and 0.4% for the 1-6 years' age group, and significant differences were obtained between the age groups (p<0.001, for all).

Ten thousand nine hundred six subjects (35.8) were in high SES, 16,641 (61,1%) was in medium SES, and 937 (3.1% ) was low SES. When we compared the metabolite levels according to SES, in 1-6 years of age; the Vitamin B12 and MCV levels were different between high and medium SES (p=0.004, p=0.019, respectively), RDW was different between low and high SES (p=0.034). In 7-12 years of age, ferritin, Hgb, RDW and Vitamin B12 levels

**Table 1.** Complete blood counts (Hb, Hct, MCV, RDW), serum folate, vitamin B12 levels and the iron status parameters (iron, iron binding capacity, ferritin) in children and adolescence. Each value represents mean±SD

| | Girls | | | | | Boys | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Age range (years) | Lower-Upper Limit | Mean±SD | Median (IQR) | Lower-Higher 95% CI | Age range (years) | Lower-Upper Limit | Mean±SD | Median (IQR) | Lower-Higher 95% CI |
| **Hemoglobin (g/dL)** | 1-6 n=751 | 32-213 | 12.3±0.9 | 12.3 (1.2) | 12.2-12.3 | 1-6 n=759 | 7.7-15.2 | 12.2±1.0 | 12.3 (1.2) | 12.2-12.3 |
| | 7-12 n=873 | 17-356 | 12.8±0.9 | 12.8 (1.1) | 12.7-12.8 | 7-12 n=923 | 8.1-15.5 | 12.9±0.9 | 12.9 (1.2) | 12.8-12.9 |
| | 13-18 n=2229 | 26-393 | 12.6±1.2* | 12.7 (1.4) | 12.6-12.7 | 13-18 n=1483 | 8.2-18.3 | 14.5±1.2 | 14.6 (1.5) | 14.5-14.6 |
| **Hematocrit (%)** | 1-6 n=751 | 80-270 | 36.7±2.6 | 36.7 (3.2) | 36.5-36.9 | 1-6 n=759 | 25-47.4 | 36.4±2.7 | 36.6 (3.6) | 36.2-366 |
| | 7-12 n=873 | 85-306 | 38.4±2.4 | 38.4 (3.1) | 38.2-38.5 | 7-12 n=923 | 27.9-45.8 | 38.4±2.4 | 38.4 (3.3) | 38.2-38.5 |
| | 13-18 n=2229 | 81-330 | 38.4±2.9* | 38.6 (3.5) | 38.3-38.5 | 13-18 n=1483 | 29.2-52.4 | 43.0±3.1 | 43.3 (4.1) | 42.9-43.2 |
| **MCV (fL)** | 1-6 n=751 | 36.6-193 | 77.4±4.9* | 77.8 (5.7) | 77-77.7 | 1-6 n=759 | 51.9-95.8 | 76.1±5.0 | 76.9 (5.5) | 75.7-76.4 |
| | 7-12 n=873 | 24.4-233.6 | 79.6±4.6* | 80 (5.4) | 79.3-79.9 | 7-12 n=923 | 53.9-94.2 | 78.8±4.9 | 78.8 (5.2) | 78.3-78.9 |
| | 13-18 n=2229 | 12.8-246 | 83.3±5.9* | 84.1 (6.3) | 83.1-83.6 | 13-18 n=1483 | 55.8-95.4 | 82.7±5.4 | 83.4 (5.9) | 82.4-83 |
| **RDW (%)** | 1-6 n=751 | 31-100 | 13.8±1.1* | 13.6 (1.1) | 13.7-13.8 | 1-6 n=759 | 11.8-22.1 | 14.0±1.1 | 13.8 (1.0) | 13.9-14 |
| | 7-12 n=873 | 27-98 | 13.6±0.9* | 13.3 (0.9) | 13.4-13.5 | 7-12 n=923 | 11.8-19.5 | 13.6±0.9 | 13.5 (1.0) | 13.5-13.6 |
| | 13-18 n=2229 | 25-108 | 13.7±1.5* | 13.4 (1.2) | 13.7-13.1 | 13-18 n=1483 | 11.4-26.2 | 13.5±1.0 | 13.3 (0.9) | 13.4-13.5 |
| **Iron (ng/mL)** | 1-6 n=891 | 4-200 | 67.7±33.6 | 65 (46) | 65.5-69.9 | 1-6 n=112 | 4-257 | 68.1±34.2 | 65 (44) | 65.9-70 |
| | 7-12 n=1317 | 1-218 | 73.7±33.4** | 72 (44) | 71.9-75.5 | 7-12 n=1057 | 3-215 | 71.7±33.8 | 67 (41) | 69.8-73.5 |
| | 13-18 n=3394 | 1-455 | 70.5±40.3* | 65 (54) | 69.1-71.8 | 13-18 n=1329 | 7-302 | 90.2±40.6 | 87 (54) | 88.4-92 |

**Table 1.** Continue

| | Girls | | | | | Boys | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Age range (years)** | **Lower-Upper Limit** | **Mean±SD** | **Median (IQR)** | **Lower-Higher 95% CI** | **Age range (years)** | **Lower-Upper Limit** | **Mean±SD** | **Median (IQR)** | **Lower-Higher 95% CI** |
| **Ferritin (ng/dL)** | 1-6 n=914 | 2.4-117.1 | 23.3±15.4 | 19.7 (17.1) | 22.3-24.3 | 1-6 n=112 | 2.8-140.7 | 22.7±16.0 | 18.6 (15.2) | 21.6-23.6 |
| | 7-12 n=1358 | 1.2-169.2 | 22.6±15.1* | 19.1 (14.8) | 21.8-23.4 | 7-12 n=1057 | 1.7-392.9 | 25.8±18.5 | 21.8 (16.7) | 24.8-26.8 |
| | 13-18 n=3608 | 0.8-860.9 | 17.6±20.9* | 13.4 (14.6) | 16.9-18.3 | 13-18 n=1329 | 1.7-528 | 34.4±25.8 | 28.5 (25.9) | 33.3-35.5 |
| **Iron Binding Capacity (µg/dL)** | 1-6 n=743 | 140-912 | 305.1±62.8 | 300 (70.5) | 300.6-309.8 | 1-6 n=112 | 51-661 | 306.2±57.9 | 304 (73) | 302.2-310.2 |
| | 7-12 n=1165 | 133-678 | 309.5±58.7** | 307 (72) | 306.1-312.8 | 7-12 n=1057 | 121-550 | 301.8±51.5 | 302 (68) | 298.8-304.8 |
| | 13-18 n=3001 | 48-716 | 321.7±73.9* | 316 (97) | 319.1-324.3 | 13-18 n=1329 | 18-571 | 286.8±63.5 | 285 (81) | 283.8-289.8 |
| **Folate (ng/mL)** | 1-6 n=234 | 3.9-23.5 | 11.2±4.1 | 10.5 (4.4) | 10.6-11.7 | 1-6 n=112 | 3.8-23.8 | 11.5±4.4 | 11 (5.8) | 11-12 |
| | 7-12 n=441 | 3.3-23.3 | 9.4±3.2 | 8.6 (4.1) | 9.1-9.7 | 7-12 n=1057 | 2.4-23.3 | 9.5±3.4 | 8.9 (4.8) | 9.2-9.8 |
| | 13-18 n=1313 | 1.9-21.8 | 6.9±2.6* | 6.5 (3) | 6.7-7 | 13-18 n=1329 | 1.3-19.9 | 6.4±2.5 | 5.9 (3.2) | 6.3-6.6 |
| **Vitamine B12 (pg/mL)** | 1-6 n=948 | 67-948 | 291.5±126.9** | 269 (150) | 283.5-299.6 | 1-6 n=112 | 64-1038 | 279.5±130.0 | 250 (158) | 271.6-287.5 |
| | 7-12 n=1493 | 53-844 | 237.9±101.8 | 218 (116) | 232.8-243.1 | 7-12 n=1057 | 57-1449 | 246.3±116.6 | 226 (129) | 240.4-252.2 |
| | 13-18 n=4081 | 51-1420 | 192.5±103.9* | 170 (89) | 189.3-195.7 | 13-18 n=1329 | 54-993 | 178.3±84.8 | 158.5 (84.3) | 174.9-181.6 |

*: p<0.001, **: p<0.05

altered significantly, (p=0.029, p=0.001, p=0.002, p<001) between high and medium SES, and however the differences in Hgb and Vitamin B12 levels were also different between medium and low SES (p=0.020, p<0.001) together with Hct. However, beside Hgb, Vitamin B12 and ferritin (p=0.006, p=0.001 for Vit-B12 and ferritin), serum iron, TIBC levels showed a significant difference between high and medium SES (p<0.001 for both). The variables affecting the risk of iron and vit-B12 deficiencies were evaluated using binary logistic regression analysis and are presented in Table 2. Girls had a 2.8-fold increased higher risk compared with boys, and increased age was associated with a 2.0-fold higher risk associated with iron deficiency. For vit-B12 deficiency, boys had a 1.2-fold higher risk, and increased age was associated with a 1.8-fold higher risk in the 7-12 years' age group; however, the risk increased 5.9-fold in the 13-18 years' age group. For folate deficiency, boys had a 1.5-fold higher risk, and increased age was associated with a 20.7-fold higher risk in 13-18 years' age group; however, the risk was 1.8-fold for the 7-12 years' age group. No associations were obtained regarding SES and iron and folate deficiencies. Only lower SES was associated with a 1.4-1.8 times higher risk for vit-B12 deficiency.

**Table 2:** Evaluation of factors associated with the risk of iron deficiency anemia (IIA) and serum vit-B12 (IIB)

**Table 2A:** Predict independent variables for the iron deficiency anemia

| | | | | | | 95% CI | |
|---|---|---|---|---|---|---|---|
| | **B** | **Wald** | **df** | **P** | **Odds ratio** | **Lower** | **Upper** |
| Age groups | | | | | | | |
| 1-6 years (reference) | | | | | | | |
| 7-12 years | -0.796 | 40.451 | 1 | <0.001 | 0.451 | 0.353 | 0.577 |
| 13-18 years | 0.742 | 78.341 | 1 | <0.001 | 2.099 | 1.781 | 2.474 |
| Gender | | | | | | | |
| Male (reference) | | | | | | | |
| Female | 1.015 | 67.100 | 1 | <0.001 | 2.759 | 2.164 | 3.518 |
| Socioeconomic status | | | | | | | |
| A (reference) | | | | | | | |
| B | 0.034 | 0.075 | 1 | 0.7840 | 1.035 | 0.81 | 1.32 |
| C | 0.091 | 0.157 | 1 | 0.6918 | 1.095 | 0.698 | 1.719 |

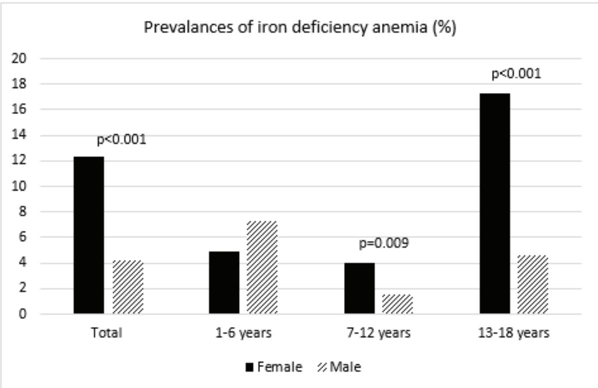
**Table 2B:** Predict independent variables for the vitamin B12 deficiency

| | | | | | | %95 CI | |
|---|---|---|---|---|---|---|---|
| | **B** | **Wald** | **df** | **P** | **Odds ratio** | **Lower** | **Upper** |
| Age groups | | | | | | | |
| 1-6 years (reference) | | | | | | | |
| 7-12 years | 0.576 | 84.633 | 1 | <0.001 | 1.779 | 1.574 | 2.012 |
| 13-18 years | 1.774 | 950.833 | 1 | <0.001 | 5.896 | 5.267 | 6.600 |
| Gender | | | | | | | |
| Female (reference) | | | | | | | |
| Male | 0.144 | 12.469 | 1 | <0.001 | 1.154 | 1.06 | 1.25 |
| Socioeconomic status | | | | | | | |
| A (reference) | | | | | | | |
| B | 0.300 | 50.385 | 1 | <0.001 | 1.350 | 1.243 | 1.467 |
| C | 0.561 | 19.371 | 1 | <0.001 | 1.753 | 1.365 | 2.250 |

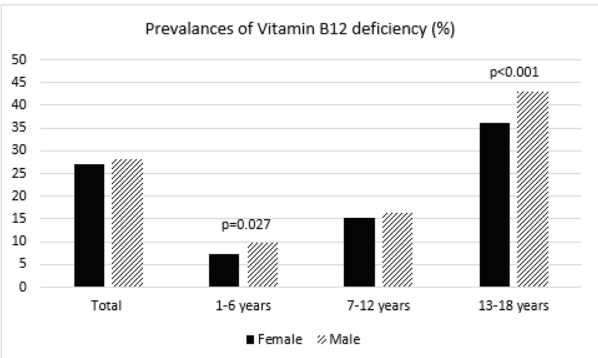
Hosmer&Lemeshow Model $\chi^2$ (6)=36.822, p<0.001 for iron deficiency and $\chi^2$ (6)=13.633, p=0.034 for vit-B12.
Variable(s) entered on step 1: Age groups, gender and socio-economic status.

Correlation analyses were conducted in the children and significant correlations between age and the following parameters: Hb (r=0.535, p<0.001), Hct (r=0.409, p<0.001), MCV (r=0.535, p<0.001), RDW (r=-0.142, p<0.001). Additionally, age was correlated with folate (r=-0.480, p<0.001), vit-B12 (r=-0.377, p<0.001). Significant correlations also existed between Hb and MCV (r=-0.393, p<0.001), Hb and RDW (r=-0.374, p<0.001).

(A)




(B)




(3)

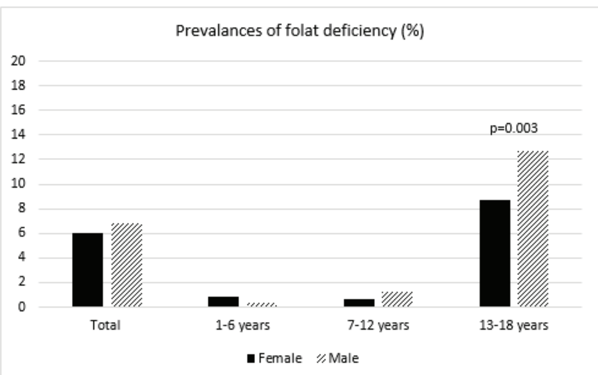



**Figure 1:** Prevalence of (A) iron deficiency (B) Vit-12 deficiency (C) folate deficiency in Turkish children aged 1-18 years. Mann Whitney U test was used for significance; p<0.05.

## DISCUSSION

Folate and vit-B12 are essential throughout life, especially in childhood and adolescence because many metabolic, hormonal, and developmental changes take place physiologically during these periods. The reference values of childhood and adolescence due to hormonal changes, healthy growth, and metabolic roles in those periods are of great importance.

In this study, blood concentrations of serum vit-B12, folate, iron, and ferritin were measured to establish reference intervals in children and adolescents aged 1-18 years. Although ferritin concentrations are recommended as the single best indicator to evaluate the body's total iron status by the WHO, interfering factors such as inflammation that cause falsely increased ferritin results should not be neglected (12). In the present study, the Hb, Hct, MCV, and RDW levels were found significantly different across the age groups in girls and boys. The iron, TIBC, and ferritin levels were also lower in girls aged in 7-12 and 13-18 years' age groups compared with the same age groups of boys. Consequently, boys aged 13-18 years have higher Hb and Hct levels than girls, and the prevalence of iron deficiency in girls was higher than in boys in this age group (17.3% vs. 4.6%), However, the girls aged 7-12 years also had higher iron deficiency rates than the same age group of boys depending on the beginning of menarche. The ferritin levels of girls showed an approximately 22% decrease in the 13-18 years' age group compared with the 7-12 years' age group, and an approximately 30% increase was seen in boys between the same age groups. In Spanish and South-East Asian adolescents, a higher prevalence of iron deficiency was reported compared with our results (13, 14). Studies of Turkish children reported that the iron deficiency rate was between 15.7% and 28%, higher in girls than in boys; however, the rate was higher in boys compared with girls early in life (15, 16). According to our findings, the lower ferritin levels were obtained compared to the results of the National Health and Nutrition Examination Survey (NHANES), and Korean NHANES (2010-2012) (17, 18). In the Korean NHANES study, the higher ferritin results were reported in boys than girls in all age groups, in accordance with our findings (18). In general, adolescents have a greater risk of iron deficiency due to an increased requirement for iron, a poor dietary intake of iron, a high rate of infection, and malabsorption due to parasitosis of the gastrointestinal tract (19). The low ferritin levels in adolescent girls can be attributed to the onset of menarche, and dietary habits such as low food and/or energy intake compared with boys. When we evaluated the iron and ferritin levels depending on the SES levels, the iron and ferritin levels of children from the highly developed region were found significantly higher compared with those of medium developed regions.

According to our study results, we found significant age-related decreases in serum vit-B12 and folic acid levels, and these alterations in both vitamin levels may be associated with the higher requirements of vitamins due to the increased metabolic demands of growing and developing children (20). There are previous studies supporting the relation of accelerated metabolism with lower vit-B12 and folate levels and less intake of folate-rich foods in the adolescence period (5). Therefore, similar age and sex-related declines in vit-B12 and folate levels were also reported by a previous study (21). In our study, the serum vit-B12 levels of girls were higher than boys in the 1-6 years' and 13-18 years' age groups (5), however, the serum folate levels of girls were only higher in the 13-18 years' age group compared with their peer group. But the results of the other age groups were similar (5). Although the folate levels obtained in our study were in agreement with the results of previous studies, we obtained lower vit-B12 levels in Turkish children than their peers; higher serum vit-B12 levels have been reported, especially from European-based population studies (5, 22-24). However, similar serum vit-B12 results were reported in a study from Turkey (25).

Studies reported that folate deficiency due to a decreased intake was seen more frequently in developing and socioeconomically distressed countries (26). When we investigated the effect of SES on vit-B12 and folate concentrations, it was found that the risk of vit-B12 deficiency increased 1.8 times with decreased SES. However, no significant effects on folate levels were observed. Similarly, the serum vit-B12 levels of children from highly developed regions were significantly higher than those of middle and poorly developed regions.

According to our results, the prevalence of vit-B12 deficiency was 27.1% and 28.3%, and 6.0% and 6.8% for folate, girls and boys, respectively. The highest deficiency rate for vit-B12 was obtained in the 13-18 years' age group with 36.1% and 43.1% in girls and boys, and the highest rate for folate deficiency was 12.7% in boys during adolescence. Studies reported that the folate deficiency rate was 15% at cut-off levels of 4.5 ng/mL for adolescents (22, 23). Also, studies documented similar or higher deficiency rates for vit-B12 compared with our findings (24-27). In a cross-sectional study performed in urban and rural parts of Turkey in adults, the prevalence of vit-B12 and folate deficiency were reported as 27.4% and 4.6%, the highest being in the southern part and the lowest in western parts of Turkey (28). These changes were associated with socioeconomic factors and regional dietary habits in different regions of Turkey (28).

In some countries, mandatory folate and vit-B12 fortification is recommended to support the metabolic effects of these vitamins. Vit-12, Hcy and folate status are considered to be important for the follow-up of cardiovascular disease development and Hcy is an independent risk factor. In our previous study, strong associations were reported between vit-B12 and folate deficiencies and hypertension (28). In North America, studies showed that the fortification of flour with folic acid made a significant contribution to public health, and decreased mortality rates were reported due to complications of CVD, especially in subjects with preexisting CVD or renal disease (29-31).

This study has some limitations and strengths. The strengths of our study are its large sample size with almost equal numbers of both sexes in all age groups. Nevertheless, the lack of detailed information about subjects including the weight, height, and nutritional habits are the most important limitations of the study.

## CONCLUSION

The results of this study are important in that the monitorization of serum ferritin, vit-B12, and folate levels greatly contribute to clinical practice because of the roles of vit-B12, folate, and iron in mental, emotional, and metabolic development. It is also of great value to monitor whether dietary intakes of vit-B12 and folate are at the required level to protect future generations from CVDs. In conclusion, mandatory fortification of cereals with iron, folic acid, and/or vit-B12 might be beneficial for public health in preventing the deficiencies of these nutrients and intercepting their adverse effects on general health.

**Ethics Committee Approval:** This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 21.02.2020, No: 2020/311).

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- H.E., S.G. E.A.; Data Acquisition- H.E., M.K., E.A.; Data Analysis/Interpretation- S.G., H.E., E.A., M.K.; Drafting Manuscript- S.G., E.A., H.E., E.A., M.K.; Critical Revision of Manuscript- E.A., S.G.; Final Approval and Accountability- S.G., E.A., H.E., E.A., M.K.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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