The prevalence of low serum levels of Vitamin D, Vitamin B12, folate and ferritin in adolescents: Single center experience

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Abstract

Background: In this study, the aim was to evaluate the prevalence of vitamin D, vitamin B12, ferritin, and folate deficiencies in adolescence to clarify the need for early diagnosis and therapy.

Methods: The medical records of adolescents between 10 and 18 years of age between 01 September 2018 and 28 February 2019 as healthy with non-specific complaints, or due to well-child care visits, were analyzed retrospectively.

Results: A total of 1847/2507 (73.6%) adolescents were included in the study. The prevalence of vitamin D deficiency was 25.7% (n: 178/691). Vitamin B12 deficiency prevalence was 69.2% (n: 753/1088). The prevalence of anemia and ferritin deficiency was 4.8% and 13.26%. The prevalence of folate deficiency was 37.9% (n: 413/1088). VDD prevalence was statistically significantly higher in females than males (F/M:116/62). VB12D prevalence, the number and mean age of females with hemoglobin deficiency, and low ferritin levels was found to be statistically significantly higher in females than males.

Conclusions: The prevalence of vitamin D, vitamin B12, folate deficiency and low ferritin levels was found to be high among adolescents. In particular, adolescents admitting with non-specific complaints and for control purposes in big cities must be considered to be at risk for the deficiency of these vitamins and low level of ferritin.

Keywords
Adolescent, vitamin D, vitamin B12, folate, ferritin

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Introduction

Adolescence is a period of life usually known with good health. Parents take their infants and toddlers to well-child visits at regular intervals. When individuals admit to the doctor at older ages with numerous health problems, their follow-up care is also performed in real time. Adolescents and even young adults do not admit or are not taken to the doctor because of the idea that they are already healthy. Thus, many of the important and preventable causes of mortality and morbidity, whose foundations are laid during these stages, are overlooked. As a result, the individual fights with both the disease and its complications in the later stages of his/her life. For this purpose, providing preventive healthcare services to adolescents is important both for the adolescents and public health. Adolescents must take advantage of preventive healthcare services at least once in each of the early, middle, and late stages of adolescence. In these follow-ups, not only training and counselling services but also screenings are recommended. Iron loss increases in females with menarche, while the need for iron increases in males with the increase in muscle mass. As a result, iron deficiency anemia can be frequently observed in both sexes. Screening for anemia via complete blood count is important in adolescence. Screening for other vitamins, however, is not recommended in adolescent follow-ups.

Due to the changes in lifestyle, social life, dietary style, dietary habits, and reduced sunlight exposure, individuals in adolescence cannot meet their micronutrient requirements. As a result, the probability of having various micronutrient deficiencies might increase. It is usually not difficult for clinicians to diagnose vitamin deficiencies in the presence of specific symptoms. However, in the presence of non-specific, general symptoms, vitamin deficiencies, or anemia might be missed. In this study, the aim was to evaluate the prevalence of vitamin D, vitamin B12, ferritin, and folate deficiencies in healthy adolescents with no organic or chronic diseases, admitting with non-specific complaints such as listlessness, fatigue, loss of appetite, mild muscle pain, and headache.

Materials and methods

The study included adolescents between 10 and 18 years old who were admitted to Gazi University Faculty of Medicine Hospital, General Pediatrics Outpatient Clinic between 01 September 2018 and 28 February 2019 with non-specific complaints or for healthcare follow-up purposes. Adolescents suffering from chronic diseases, adolescents who are currently taking or have recently taken vitamin supplements and adolescents previously diagnosed with vitamin deficiency were excluded from the study. Listlessness, fatigue, loss of appetite, headache, and feeling unwell were considered as non-specific complaints. In addition to these cases, adolescents with no complaints whose parents claimed that they were not eating healthily were also evaluated. The cases’ files were retrospectively analyzed after the necessary local permissions were obtained. In addition, depending on the clinician’s suspicions of deficiency based on the adolescents’ complaints, the results of
ferritin, vitamin D, vitamin B12, and folate studies were also recorded. In the study, having hemoglobin levels <12 mg/dl was considered as anemia, having Vitamin B12 levels <200 pg/ml as deficiency, folic acid levels <4 ng/ml as deficiency, vitamin D levels <15 g/l as deficiency, and ferritin levels <12 g/l as deficiency. The data were transferred to and analyzed using Statistical Package for the Social Sciences (SPSS version.22) statistics package software, and contingency tables, $\chi^2$, correlation, and percent distribution analyses were used.

This study was performed with the approval of the Gazi University Ethics Committee (Project no. 2019-3).

**Results**

Within 6 months, 2507 adolescents were admitted to General Pediatrics outpatient clinics. A total of 1847 (73.6%) adolescents were included in the study. Complete blood count of 1847 adolescents, ferritin study of 1073 adolescents (58%), vitamin D study of 691 adolescents (37.4%), and B12 and folate study of 1088 adolescents (58.9%) were performed. The mean age was 13.55 ± 2.3 years. There were 853 males and 994 females, and the mean ages by gender were 13.14 ± 1.2 and 13.84 ± 0.9 years for males and females, respectively. Vitamin B12 deficiency (VB12D) prevalence was 69.2% (n: 753/1088) based on the cut-off value of <300 pg/ml whereas it was 38.3% (n: 417/1088) based on the cut-off value of <200 pg/ml. The prevalence of hemoglobin deficiency was 4.8% (n: 89/1847). The prevalence of ferritin deficiency was 13.26% (n: 245/1847). The prevalence of folate deficiency was 37.9% (n: 413/1088). None of the adolescents included in the study group were on a specific diet (vegan, vegetarian, etc.). Moreover, in this retrospective evaluation since the way of dressing was not included in the medical information of the females included in the study, it could not be evaluated.

It was found that 29.2% of 1847 adolescents included in the study did not have any complaints. The most common complaints among the remaining 70.8% were listlessness and fatigue, followed by, in decreasing order, loss of appetite, feeling unwell, attention deficiency, headache, reluctance to study, getting tired quickly, and muscle-extremity pain. All adolescents were attending school. The percentage of adolescents regularly doing sports was 4.3%. None of the patients included in the study were previously diagnosed with anemia or vitamin deficiency. It was found that no vitamin or iron supplements were given to these adolescents in the last 2 years. None of the adolescents had obesity or malnutrition.

When all adolescents were classified into three subgroups based on 3-year-periods between ages 10 and 18, no statistically significant difference was found between the groups in terms of blood levels and distribution of the number of cases. When evaluated in terms of gender, no statistically significant difference was detected in terms of distribution by gender and vitamin D, vitamin B12, ferritin, and folate levels (Table 1). It was found that the most frequently admitted group was 10–12 years age group (38%).
|                                | 10–18 years | 10–12 years | 13–15 years | 16–18 years | M/F       | p Value |
|--------------------------------|-------------|-------------|-------------|-------------|-----------|---------|
| Ferritin (n)                   | 1073        | 381         | 406         | 286         | 448/625   | >0.05   |
| Ferritin*                      | 27.5 ± 23.6 | 28.1 ± 19.5 | 23.8 ± 18.1 | 31.8 ± 30.7 | 25.5 ± 20.2/29.3 ± 23.1 | >0.05   |
| Vitamin D (n)                  | 692         | 241         | 253         | 197         | 304/388   | >0.05   |
| Vitamin D*                     | 21.5 ± 9.44 | 22.6 ± 8.1  | 21.1 ± 10.5 | 20.7 ± 9.2  | 22.1 ± 8.05/21.08 ± 10.3 | >0.05   |
| Vitamin B12 (n)                | 1088        | 373         | 419         | 296         | 464/624   | >0.05   |
| Vitamin B12*                   | 273.5 ± 171.5 | 301.3 ± 156.3 | 254.3 ± 157.2 | 265.7 ± 202.5 | 275.6 ± 162.1/282.4 ± 158.2 | >0.05   |
| Hemoglobin (n)                 | 1847        | 703         | 661         | 483         | 853/994   | >0.05   |
| Hemoglobin*                    | 14.01 ± 1.3 | 13.6 ± 0.9  | 14.08 ± 1.4 | 14.4 ± 1.6  | 13.9 ± 1.2/14.2 ± 0.8 | >0.05   |
| Folate (n)                     | 1088        | 373         | 419         | 296         | 464/624   | >0.05   |
| Folate*                        | 6.2 ± 1.2   | 6.4 ± 1.1   | 5.9 ± 0.9   | 6.3 ± 1.2   | 6.3 ± 1.1/6.1 ± 0.8 | >0.05   |

*Mean ± standard deviation.
Complete blood count was performed for all 1847 adolescents. It was most frequently performed in 10–12 year-olds. When classified according to age and gender, no statistically significant intragroup difference was found. The mean hemoglobin level of the group was 14.01 ± 1.3 g/dl, while it was 13.9 ± 1.2 g/dl for the males and 14.2 ± 0.8 g/dl for the females. The frequency of adolescents with hemoglobin level <12 g/dl was 4.8% (n: 89/1847). Of these adolescents, 18 were male and 71 were female, and the mean ages were 12.05 ± 2.01 years and 14.5 ± 1.9 years, respectively. Although there was no statistically significant difference between the two groups in terms of levels, there was a statistically significant difference in terms of age and sex distribution (p < 0.05). Ferritin levels were studied in 1073 adolescents (58%). Unlike hemoglobin, it was most frequently studied in 13–15-year-olds. The frequency of low ferritin levels was 13.26% (n: 245/1847), the mean age was 14.2 ± 2.09 years and the mean ferritin level was 8.02 ± 2.79 g/l. In the group with low ferritin levels, there were 34 males and 211 females, the mean age was 12.8 ± 1.98 years in males and 14.5 ± 2.02 years in females, and the mean serum level was 9.05 ± 2.43 ng/ml in males and 7.85 ± 2.8 ng/ml in females. Similar to the low hemoglobin group, the number and mean age of females in the low ferritin group were statistically significantly higher than the males.

In 692 (37.4%) adolescents whose serum vitamin D levels were studied, the mean level was 21.5 ± 9.44 μg/l. Serum vitamin D levels were most frequently studied in 13–15-year-olds (253/697), with the mean serum level of 21.1 ± 10.5 μg/l. There were 304 males and 388 females, and the mean serum vitamin D levels were 22.1 ± 8.05 μg/l in males and 21.08 ± 10.3 μg/l in females. There were no statistically significant differences between the three age groups in terms of the number of adolescents and serum levels, and distribution by gender and serum vitamin D levels by gender. Vitamin D deficiency (VDD) prevalence in adolescents was 25.7% (n: 178/692). There were 62 males and 116 females, and the mean serum vitamin D level was 12.5 ± 1.9 μg/l in males and 12.3 ± 1.9 μg/l in females, with mean ages of 13.4 ± 2.2 and 14.4 ± 2.1 years, respectively. The prevalence of VDD in females was statistically significantly higher than in males (M/F: 62/116). There were no statistically significant differences between genders in terms of age and levels.

Vitamin B12 and folate levels were analyzed in 1088 (58.9%) adolescents. Both vitamin B12 and folic acid levels were most frequently studied in 13–15 year-olds (419/1088 = 38.5%). The mean serum vitamin B12 level of 1088 adolescents was 273.5 ± 171.5 pg/ml, and the mean serum folate level was 6.2 ± 1.2 ng/ml. When analyzed according to whole-group and distribution by group, there were no statistically significant differences between vitamin B12 and folate levels. There were 464 males and 624 females. The mean serum vitamin B12 and folate levels were 275.6 ± 162.1/282.4 ± 158.2 and 6.3 ± 1.1/6.1 ± 0.8, respectively. There were no statistically significant differences between serum B12 and folate levels between all groups, subgroups, and genders. Vitamin B12 deficiency (VB12D) prevalence was 69.2% (n: 753/1088) based on the cut-off value of <300 pg/ml whereas it was 38.3% (n: 417/1088) based on the cut-off value of <200 pg/ml. Based on the cut-off value of <300 pg/ml, vitamin B12 deficiency prevalence was found to be
significantly higher in females than males (284/469). No statistically significant difference was detected between the three groups in terms of age. No statistically significant difference was detected within the cut-off subgroups in terms of serum levels and age according to gender. Folate deficiency was found in 413 adolescents. No statistically significant difference was detected in terms of gender, age, and serum levels. The distribution of deficiencies of several micronutrients in adolescents was shown in Table 2.

Discussion

In this study, the micronutrient status of adolescents admitting with non-specific symptoms or for follow-up care was evaluated. In our study, contrary to the expectations, the prevalence of anemia was found to be 4.8% and the prevalence of low ferritin levels was found to be 13.26%, although the prevalence of other micronutrient deficiencies was high in adolescents admitted mostly with non-specific symptoms.

It is recognized that adolescence is a period in which development and growth continue, and consequently, nutritional anemia is common. Anemia negatively affects cognitive performance in adolescents. In our study, anemia prevalence was 4.8%, and the prevalence of low ferritin levels was 13.26%. In NHANES (national health and nutrition examination survey) study, the prevalence of iron deficiency especially in 14–18-year-olds was reported as 4.5%. In a study performed in India on adolescent girls with anemia, it was found that two-thirds of the cases were anemic due to iron deficiency. In another study, it was reported that 15% of anemia in adolescents was due to iron deficiency, whereas 42.5% was due to vitamin B12 deficiency. In a study in China, it was reported that the prevalence of iron deficiency anemia significantly increased in children living in rural areas. This was found to be 59.7% in the India study. In our study, the prevalence of ferritin deficiency was found to be 13.26%, and as expected, it was higher than the prevalence of anemia. While approximately one in every eight adolescents in the study group still had iron deficiency, they were at risk of iron deficiency anemia. We found that all anemic patients had low ferritin levels and microcytic anemia. None of the patients had megaloblastic anemia. The lower prevalence of iron deficiency compared to the deficiency of other nutrients may be because erythropoiesis slows down in nutrient deficiency and thus, iron cannot be used. The fact that the age of females was statistically significantly higher than that of males and the statistically significantly higher prevalence of anemia and low ferritin levels in females than males can be attributed to menarche. Moreover, vitamin B12 deficiency prevalence was high in this group. Since vitamin B12 replacement would increase erythrocyte synthesis, it can cause depletion of iron and/or folic acid. However, since the patients under treatment were excluded from our study, this hypothesis cannot be used. Continuation of growth is important, especially in children. Since bone marrow production will decrease and iron cannot be used in B12 and folate deficiency, iron parameters can be relatively high. However, with the recent
| Nutrient       | n     | Age (±SD)        | Serum levels (±SD) | p Value  |
|---------------|-------|------------------|--------------------|----------|
| Ferritin*     | 245   | 14.2 ± 2.09      | 8.02 ± 2.79        | 0.05     |
| Male/female   | 34/211| 12.8 ± 1.98/14.5 ± 2.02 | 9.05 ± 2.43/7.85 ± 2.8 | >0.05 |
| Vitamin D**   | 178   | 14.06 ± 2.2      | 12.4 ± 2.02        | 0.05     |
| Male/female   | 62/116| 13.4 ± 2.2/14.4 ± 2.1 | 12.5 ± 1.9/12.3 ± 1.9 | >0.05 |
| Hemoglobin*** | 89    | 14.02 ± 2.2      | 11.1 ± 0.8         | 0.05     |
| Male/female   | 18/71 | 12.05 ± 2.01/14.5 ± 1.9 | 11.4 ± 0.5/11.1 ± 0.92 | >0.05 |
| Vitamin B12   |       |                  |                    |          |
| <300 pg/ml    | 753   | 13.8 ± 2.2       | 190.4 ± 59.02      | 0.05     |
| Male/female   | 284/469| 14.01 ± 1.9/13.7 ± 2.1 | 185.5 ± 49.9/192.4 ± 56.8 | >0.05 |
| <200 pg/ml    | 417   | 14.1 ± 2.1       | 145.9 ± 34.5       | 0.05     |
| Male/female   | 176/241| 14.2 ± 1.5/13.9 ± 1.1 | 141.5 ± 29.4/147 ± 44.2 | >0.05 |
| <150 pg/ml    | 220   | 14.2 ± 2.03      | 119.3 ± 23.9       | 0.05     |
| Male/female   | 92/128| 14.4 ± 1.8/14.04 ± 1.7 | 123 ± 33.1/115.25 ± 29.5 | >0.05 |
| Folate****    | 413   | 13.2 ± 1.9       | 1.6 ± 0.3          | 0.05     |
| Male/female   | 187/226| 13.04 ± 1.1/13.4 ± 0.5 | 1.5 ± 0.2/1.7 ± 0.1 | >0.05 |

*Patients with serum ferritin levels < 12 ng/ml.
**Patients with serum Vitamin D levels < 15 μg/l.
***Patients with hemoglobin levels < 12 g/dl.
****Patients with folate levels < 4 ng/ml.
presence of iron supplements in industrial products, this condition can be explained. It can lead to an absence of iron deficiency although it is expected nutritionally. However, as a result of the small number of products supplemented with micronutrients such as vitamin B12, the adolescent, who already has poor nutritional intake, cannot obtain the micronutrients from other processed products.

In studies performed in numerous countries with healthy adolescents or adolescents at risk for various diseases, vitamin D deficiency was reported. In the study by Yetim et al. on adolescents, the prevalence of vitamin D deficiency (≤20 ng/ml) was reported as 56%. It was shown that the prevalence of vitamin D deficiency increases especially in obese adolescents. The causes behind this were reported as sedentary lifestyle and the reluctance to go outside. In our study, the prevalence of vitamin D deficiency was found to be 25.7%. In the study by Gonzalez-Groos et al. on 1006 adolescents, the prevalence of vitamin D deficiency was found to be 15%. Dong et al. reported the prevalence of vitamin D deficiency in children and adolescents as 28.8%. In our study group, there were no obese adolescents or adolescents with malnutrition. It was found that vitamin D deficiency prevalence was higher than the prevalence of anemia. Although the prevalence seemed lower than other studies, our study was performed in a larger population with a more balanced age distribution. Moreover, in our study, the cut-off value for vitamin D deficiency was 15 μg/l, which was higher than other studies, thus resulting in a lower prevalence. The causes of this vitamin D deficiency prevalence among adolescents can be attributed to sedentary lifestyle, inadequate physical activity, and their willingness to spend time indoors. Moreover, the prevalence of vitamin D deficiency was found to be statistically significantly higher in females. While the prevalence was also higher in females in the study performed in Pakistan, it was found to be higher in males in the study performed in Norway. These differences were mostly attributed to cultural differences (way of dressing, diet, etc.). In a previous study performed in our country, it was shown that vitamin D levels were lower in adolescents with a more modest way of dressing. However, since our study was retrospectively designed, vitamin D levels depending on the way of dressing could not be analyzed. In any case, the significantly high prevalence of vitamin D deficiency among adolescents in our society was striking. It is important to study the level of this vitamin in clinical practice.

Clinical vitamin B12 deficiency manifesting with hematological and neurological symptoms is not common in the general population. However, in the absence of significant hematological and neurological symptoms, low or marginal vitamin B12 status is observed very frequently among the population. This was attributed to low-level consumption of animal source foods rich in vitamin B12. The prevalence of vitamin B12 deficiency (VB12D) was 69.2% (n: 753/1088) when a cut-off value of <300 pg/ml was used whereas it was 38.3% (n: 417/1088) when a cut-off value of <200 pg/ml was used. It was found that there were 220 adolescents (20.2%) when a cut-off value of <150 pg/ml was used. The prevalence of vitamin B12 deficiency (<300 pg/ml) in the study performed by Yetim et al. on adolescents was reported as 35.6%. It was reported as 73.5% in the study performed in
India. In the NHANES study, the prevalence of low vitamin B12 levels were 2.9% and 10.6% when cut-off values <148 and 200 pg/ml were used, respectively.

In our study, the adolescents mostly had non-specific symptoms. Yetim et al. found that vitamin B12 deficiency did not cause any clinical symptoms in adolescents. Similarly, in our study, we found that common complaints were dominating whereas classical hematological and neurological symptoms were absent. In particular, a disorganized lifestyle and irregular and imbalanced dietary habits of adolescents can cause the deficiency of numerous micronutrients. In addition to vitamin B12 deficiency, we also found the prevalence of folate deficiency as 37.9%, which was similar to the prevalence of vitamin B12 deficiency. The prevalence of folate deficiency among adolescents in India was 39.8%, which was also similar to our study.

The problem that needs emphasis is that why iron deficiency or iron deficiency anemia prevalence was lower while vitamin B12 and folate deficiency prevalences were higher among adolescents despite the same source of intake. Many causes such as the source of intake, differences in ingredients of processed foods with supplements, dietary habits can be suggested. Our study has shown that various micronutrients must be studied in adolescents in addition to low ferritin levels and iron deficiency anemia. The deficiency will be higher in those with more complaints. In adolescents with complaints, vitamin B12 and folate studies should be considered. Further studies are required.

**Conclusion**

In adolescents, the prevalence of vitamin D, vitamin B12, and folate deficiencies and low ferritin levels were found to be high. This can be attributed to the use of readymade food products supplemented with iron, thus causing decreased intake from a normal diet and resulting in more significant vitamin B12, folic acid, and vitamin D deficiencies. In addition to foods supplemented with iron, other vitamins should be considered. Adolescents must be encouraged to adopt healthy dietary habits. Adolescents admitting with non-specific complaints and for control purposes in big cities, in particular, must be considered to be at risk for the deficiency of these vitamins and low ferritin levels, and their levels should be evaluated.

One of the limitations of this study was that the nutritional characteristics of adolescents were not recorded in their medical records. Further studies are needed to determine the causes of high vitamin B12 deficiency compared to anemia.

**Implications for school health**

As is known, anemia is more common in adolescence. Unlike other age groups, adolescents present with non-specific findings. Non-specific findings may not be considered as an important marker in an adolescent follow-up. In adolescent follow-ups, attention should be paid to micronutrient deficiencies as well as anemia.
Especially vitamin B12 and folate deficiency, which may affect school success and continuity.

Acknowledgement
I would like to thank Prof. Deniz Aslan for her support in preparing the manuscript.

Author contributions
NMK: Conceptualization, methodology, formal analysis, data curation, writing, and review.

Declaration of conflicting interests
The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author received no financial support for the research, authorship, and/or publication of this article.

Ethical approval
This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the University Institutional Review and Ethical Board (Project no. 2019-369).

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