

# Retrospective Investigation of the Relationship between Serum Vitamin B12 Levels and Thyroid-stimulating Hormone Levels in Adolescents

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## ABSTRACT

**Aim and background:** Adolescents often experience multiple vitamin and mineral deficiencies, with vitamin B12 deficiency being one of the most common. Vitamin B12 deficiency can lead to symptoms affecting various systems. Similarly, thyroid dysfunctions also manifest with overlapping symptoms, and vitamin deficiencies play a role in the development of thyroid diseases. In this study, we aimed to investigate the relationship between vitamin B12 and TSH levels.

**Materials and methods:** A retrospective analysis was conducted on adolescent patients who visited the Adolescent Clinic at Istanbul Education and Research Hospital. The study included 390 patients who met the inclusion criteria. The relationship between vitamin B12 levels and thyroid function parameters was examined.

**Results:** Of the patients, 228 (58%) were female, with a mean age of  $14.02 \pm 2.25$  years. The mean vitamin B12 level was  $305.23 \pm 117.31$  pg/mL. Among the patients, 326 (83.59%) had vitamin B12 levels above 200 pg/mL, 300 (77.12%) had TSH levels between 0.5 IU/mL and 5 IU/mL, and 152 (72.04%) had anti-TPO levels below 9 IU/mL. A statistically significant difference was found in the mean fT4 levels between the group with vitamin B12 levels <200 pg/mL and the group with levels  $\geq 200$  pg/mL ( $p < 0.05$ ).

**Conclusion:** The significant relationship identified between vitamin B12 and fT4 levels suggests a possible connection between vitamin B12 and thyroid hormones. However, further research is needed to explore this relationship in more detail.

**Clinical significance:** Many studies have been conducted in the adult age group to determine whether there is a relationship between thyroid dysfunctions, which share similar symptoms and have a high likelihood of co-occurrence, and vitamin B12 deficiency. In this study, we examined this relationship in the adolescent age group.

**Keywords:** Adolescent, Thyroid-stimulating hormone, Vitamin B12.

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## INTRODUCTION

Vitamin B12, also known as cobalamin, is a molecule with a structure containing a cobalt ion within a corrin ring. It belongs to the group of water-soluble vitamins and acts as a coenzyme in the human body.<sup>1</sup> Vitamin B12 has two critical functions in the body: DNA synthesis and maintaining the structure and functions of neurons in the nervous system.<sup>2</sup> As the human body cannot produce vitamin B12, it must be obtained through dietary sources.<sup>3</sup> This vitamin is predominantly found in animal-based foods. For vitamin B12 to fully perform its coenzyme function in a healthy individual, a daily intake of 2.5 µg is required.<sup>4</sup> Vitamin B12 deficiency is among the most common micronutrient deficiencies worldwide.<sup>5</sup> It can occur for various reasons, primarily due to insufficient intake and malabsorption.<sup>6</sup> Adolescence is one of the periods with an increased prevalence of vitamin B12 deficiency. Some studies have shown that undetected vitamin B12 deficiency in this period can negatively affect development, even if appropriately treated later.<sup>7</sup>

Another clinical condition frequently observed in the adolescent age group, with similar symptoms, is thyroid dysfunction. Particularly in patients diagnosed with hypothyroidism, complaints such as fatigue, weakness, dementia, depression, memory impairment, numbness, and tingling may occur.<sup>8,9</sup> Recent studies have increasingly highlighted a potential relationship between vitamin B12 deficiency and hypothyroidism. In one study, the prevalence of vitamin B12 deficiency among

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hypothyroid patients was found to be approximately 40%.<sup>10</sup> Most studies linking vitamin deficiencies and thyroid dysfunction have been conducted in adults. In this study, we aimed to evaluate the relationship between vitamin B12 levels and TSH levels in adolescents.

## MATERIALS AND METHODS

This study is a single-center, descriptive, cross-sectional, and retrospective study. It was conducted by retrospectively analyzing the clinical and laboratory data of adolescents who visited the

Adolescent Clinic of Istanbul Education and Research Hospital for general check-ups between March 1, 2022, and March 1, 2023. Inclusion criteria were being aged 10–19 years and having both vitamin B12 and TSH levels measured. Exclusion criteria included having undergone stomach and/or intestinal surgery, having gastritis or other stomach diseases, receiving treatment for vitamin B12 deficiency, and using medications for thyroid dysfunctions. A total of 390 participants meeting the inclusion criteria were identified. Data recorded included the patients' dates of birth, admission dates, gender, and laboratory values for vitamin B12, TSH, free T4 (fT4), free T3 (fT3), and anti-TPO. Statistical analyses for this study were performed using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Descriptive statistical methods (mean, standard deviation, median, interquartile range) were employed for data evaluation. The Shapiro–Wilk test was used to assess the distribution of variables. For comparisons of two groups, an independent *t*-test was applied to variables with a normal distribution, and the Mann–Whitney *U* test was used for variables without a normal distribution. Chi-square tests were employed for comparisons of categorical data. The Pearson correlation test was used to determine the relationships between variables. Linear regression analysis was conducted to assess the relationship between vitamin B12 and other variables. Results were considered significant at  $p < 0.05$ .

This thesis study was prepared in compliance with the Declaration of Helsinki, Good Clinical Practice principles, and ethical rules for research on human subjects. It was approved by the Academic Council of SBÜ Hamidiye Faculty of Medicine on May 15, 2023 (Academic Council Decision No. 39), and ethical approval was granted on June 2, 2023 (Ethics Approval No. 143).

## RESULTS

A total of 390 patients were included in the study. Of these, 228 (58%) were female, with a mean age of  $14.02 \pm 2.25$  years. The mean vitamin B12 level of the patients was  $305.23 \pm 117.31$  pg/mL. The general characteristics of the patients are presented in Table 1.

The mean vitamin B12 level was  $320.67 \pm 132.12$  pg/mL in males and  $294.26 \pm 104.47$  pg/mL in females. No statistically significant difference was observed between the mean vitamin B12 levels of the male and female groups ( $p = 0.165$ ) (Table 2).

Among the patients, 326 (83.59%) had a vitamin B12 level above 200 pg/mL, 300 (77.12%) had a TSH level between 0.5 and 5 IU/mL, and 152 (72.04%) had an anti-TPO level below 9 IU/mL (Table 3).

The patients were divided into two groups based on their vitamin B12 levels:  $\geq 200$  pg/mL and  $< 200$  pg/mL. When the two groups were compared in terms of gender, mean TSH, TSH distribution, mean fT3, mean anti-TPO, and anti-TPO distribution, no statistically significant differences were found ( $p > 0.05$ ). However, a statistically significant difference was observed in the mean fT4 levels between the group with vitamin B12  $< 200$  pg/mL and the group with vitamin B12  $\geq 200$  pg/mL ( $p < 0.05$ ) (Table 4).

A statistically significant positive correlation was observed between vitamin B12 levels and fT4 levels ( $r = 0.138$ ;  $p = 0.007$ ). A statistically significant negative correlation was observed between vitamin B12 levels and age ( $r = -0.208$ ;  $p = 0.0001$ ). No statistically significant correlation was found between vitamin B12 levels and TSH, fT3, or anti-TPO levels ( $p > 0.05$ ) (Table 5).

In the linear regression analysis, the relationship with age was found to be statistically significant in a negative direction ( $p = 0.001$ ,

**Table 1:** General characteristics of the patients

| Variable         | N   | Min  | Max  | Mean   | SD     |
|------------------|-----|------|------|--------|--------|
| Age              | 390 | 10   | 18.9 | 14.02  | 2.25   |
| B12 (pg/mL)      | 390 | 100  | 796  | 305.23 | 117.31 |
| TSH (IU/mL)      | 389 | 0.02 | 36   | 3.48   | 3.35   |
| fT4 (ng/mL)      | 388 | 0.51 | 28.7 | 11.58  | 2.70   |
| fT3 (ng/mL)      | 184 | 2.73 | 53.5 | 4.19   | 3.72   |
| Anti-TPO (IU/mL) | 211 | 0.09 | 700  | 40.87  | 114.83 |

**Table 2:** Distribution of serum vitamin B12 levels in females and males

| Gender | N   | B12 (mean $\pm$ SD) | <i>p</i> -value |
|--------|-----|---------------------|-----------------|
| Male   | 162 | $320.67 \pm 132.12$ | 0.165           |
| Female | 228 | $294.26 \pm 104.47$ |                 |

**Table 3:** Distribution of general characteristics of the patients

| Variable         | Category   | <i>n</i> = 390 | %     |
|------------------|------------|----------------|-------|
| Gender           | Male       | 162            | 41.54 |
|                  | Female     | 228            | 58.46 |
| B12 (pg/mL)      | $< 200$    | 64             | 16.41 |
|                  | $\geq 200$ | 326            | 83.59 |
| TSH (IU/mL)      | $< 0.5$    | 3              | 0.77  |
|                  | 0.5–5      | 300            | 77.12 |
|                  | $> 5$      | 86             | 22.11 |
| Anti-TPO (IU/mL) | $< 9$      | 152            | 72.04 |
|                  | $\geq 9$   | 59             | 27.96 |

**Table 4:** Comparison of parameters based on vitamin B12 levels

| Parameters                     | Category | <200 vit-B12 (n = 64) | ≥200 vit-B12 (n = 326) | p-value |
|--------------------------------|----------|-----------------------|------------------------|---------|
| Age (mean ± SD)                |          | 14.09 ± 2.23          | 14.01 ± 2.26           | 0.793   |
| Gender                         | Male     | 22 (34.38%)           | 140 (42.94%)           | 0.203   |
|                                | Female   | 42 (65.62%)           | 186 (57.06%)           |         |
| TSH (IU/mL) (mean ± SD)        |          | 3.12 ± 1.93           | 3.55 ± 3.56            | 0.646   |
| TSH (IU/mL) (Median, IQR)      |          | 2.56 (1.64–4.58)      | 2.62 (1.7–4.825)       |         |
| TSH (IU/mL)                    | <0.5     | 1 (1.56%)             | 3 (0.92%)              | 0.715   |
|                                | 0.5–5    | 51 (79.69%)           | 249 (76.38%)           |         |
|                                | >5       | 12 (18.75%)           | 74 (22.70%)            |         |
| T4 (ng/mL) (mean ± SD)         |          | 10.79 ± 4.31          | 11.73 ± 2.22           | 0.011   |
| T3 (ng/mL) (Mean ± SD)         |          | 4.04 ± 1.11           | 4.22 ± 4.02            | 0.891   |
| T3 (ng/mL) (Median, IQR)       |          | 3.81 (3.435–4.32)     | 3.85 (3.46–4.34)       |         |
| Anti-TPO (IU/mL) (Mean ± SD)   |          | 23.91 ± 51.01         | 44.6 ± 124.35          | 0.950   |
| Anti-TPO (IU/mL) (Median, IQR) |          | 5.01 (3–10.9)         | 5 (3–9.87)             |         |
|                                |          |                       |                        |         |
| Anti-TPO (IU/mL)               | <9       | 27 (71.05%)           | 125 (72.25%)           | 0.881   |
|                                | ≥9       | 11 (28.95%)           | 48 (27.75%)            |         |

**Table 5:** Pearson correlation test based on serum vitamin B12 levels

| Variable         | Statistics | Vitamin B12 |
|------------------|------------|-------------|
| Age              | <i>r</i>   | –0.208      |
|                  | <i>p</i>   | 0.0001      |
| TSH (IU/mL)      | <i>r</i>   | –0.049      |
|                  | <i>p</i>   | 0.334       |
| T4 (ng/mL)       | <i>r</i>   | 0.138       |
|                  | <i>p</i>   | 0.007       |
| T3 (ng/mL)       | <i>r</i>   | –0.01       |
|                  | <i>p</i>   | 0.895       |
| Anti-TPO (IU/mL) | <i>r</i>   | –0.064      |
|                  | <i>p</i>   | 0.351       |

$p = 0.034$ ), and the relationship with T4 was statistically significant in a positive direction ( $p = 0.034$ ) (Table 6).

## DISCUSSION

The relationship between vitamin B12 and TSH is generally attributed to the autoimmune mechanism present in the pathophysiology of autoimmune hypothyroidism and pernicious anemia. However, it may also stem from various factors, including insufficient intake or altered intestinal absorption due to slow bowel motility, intestinal wall edema, and bacterial overgrowth.<sup>11</sup> The prevalence of pernicious anemia increases with age, peaking around 60 years. In children, a condition called congenital pernicious anemia, which is histologically distinct from adult pernicious anemia due to normal gastric mucosa and acid secretion, is observed. Studies predominantly focus on adult-onset pernicious anemia, with most research conducted on adults.<sup>12</sup> Our study is unique in being the first to address this issue in the adolescent age group.

Studies conducted both globally and in Turkey have yielded highly variable results regarding the prevalence of vitamin B12 deficiency. While vitamin B12 deficiency is observed in 1–3% of children in developed countries, this rate can rise to 40–80% in developing countries.<sup>5</sup> In our study, the proportion of patients with vitamin B12 levels <200 pg/mL was 16.41% ( $n = 64$ ). A study

**Table 6:** Linear regression test for parameters with significant correlations

| Variable    | Unstandardized B | SE   | Standardized |          |          |
|-------------|------------------|------|--------------|----------|----------|
|             |                  |      | Beta         | <i>t</i> | <i>p</i> |
| Age         | –9.83            | 3.06 | –0.18        | –3.21    | 0.001    |
| fT4 (ng/mL) | 5.54             | 2.61 | 0.11         | 2.13     | 0.034    |

conducted in Europe on adolescents reported a 2% prevalence of vitamin B12 deficiency.<sup>13</sup> A study by Kefeli et al.<sup>14</sup> found vitamin B12 deficiency in 33.4% of patients. Another study conducted in Turkey<sup>15</sup> among children aged 11–18 reported a prevalence of vitamin B12 deficiency of 41.6% ( $n = 5,930$ ). In a study conducted in Diyarbakır<sup>16</sup> among individuals aged 12–22, vitamin B12 deficiency was found to be 2.2%. We believe that the wide range in these prevalence rates is influenced by variations in the reference values used for vitamin B12 deficiency, the developmental status of countries, and regional dietary habits.

In our study, vitamin B12 deficiency was identified in 18.42% ( $n = 42$ ) of females and 13.58% ( $n = 22$ ) of males, with no statistically significant difference observed between genders. Similarly, most studies in the literature do not report a gender-related difference, although one study<sup>17</sup> identified 76.6% of those with vitamin B12 deficiency as females.

When comparing the adolescent age group to early childhood, vitamin B12 deficiency has been shown to be more prevalent in adolescents.<sup>14,18–20</sup> Rasmussen et al.<sup>18</sup> found a higher prevalence of vitamin B12 deficiency in the 12–19 age group in a study involving children aged 4–19. Sahin et al.,<sup>19</sup> in a study of 64,487 pediatric patients, reported a vitamin B12 deficiency rate of 20.1%, with this rate increasing to 34.69% ( $n = 2,168$ ) in children over 12 years old. Our study also observed a statistically significant negative correlation between vitamin B12 levels and age, consistent with the literature ( $r: -0.208$ ;  $p = 0.0001$ ). Adolescence is a period of rapid growth and development when individuals gain independence in dietary habits, often leading to irregular eating patterns, skipping meals, frequent consumption of fast food, and diets high in carbohydrates. These factors may contribute to the observed increase in vitamin B12 deficiency during this period.

Studies exploring the relationship between vitamin B12 and hypothyroidism have produced varying results. Many studies have investigated whether there is an association between vitamin B12 and TSH, T4, and anti-TPO levels.<sup>12</sup> Some studies have found an inverse relationship between vitamin B12 and TSH, while others have not identified a statistically significant correlation.

Erdoğan et al.<sup>21</sup> found no statistically significant relationship between vitamin B12 and TSH in their study involving 400 adult patients. Similarly, Gökçe et al.<sup>22</sup> did not observe a significant relationship between vitamin B12 and TSH. In contrast, Aktaş et al.<sup>23</sup> in a study of 130 adult patients diagnosed with autoimmune hypothyroidism, observed significantly higher anti-TPO levels in patients with low vitamin B12 levels and a weak, negative, statistically significant correlation between anti-TPO and vitamin B12. Güler et al.<sup>24</sup> in a study of 400 participants comprising patients and a control group, found an inverse correlation between TSH and vitamin B12, a positive correlation between fT4 and vitamin B12, and an inverse correlation between anti-TPO and vitamin B12. Otlar et al.<sup>25</sup> in a study involving 40 hypothyroid patients and 30 healthy controls, identified significantly lower vitamin B12 levels in hypothyroid patients. Orzechowska-Pawilojc et al.<sup>26</sup> in a study of 61 individuals, found that the average vitamin B12 levels in hypothyroid patients were statistically significantly lower than those in the healthy group.

In our study, when comparing the TSH, fT4, fT3, and anti-TPO levels between groups with vitamin B12  $\geq 200$  pg/mL and  $< 200$  pg/mL, a statistically significant positive correlation was observed between fT4 and vitamin B12, whereas no statistically significant relationships were identified with TSH, fT3, or anti-TPO.

One potential reason for the differences among studies is that most studies rely on total serum vitamin B12 levels, which do not fully reflect the active form, holotranscobalamin. Accurate measurement of the active form requires the assessment of homocysteine and methylmalonic acid levels, which are often not evaluated. This discrepancy might contribute to the differences observed across studies.

## CONCLUSION

Vitamin B12 deficiency was identified in both male and female adolescents in our study. We suggest that annual laboratory screening for vitamin B12 deficiency during this period would be beneficial. The significant relationship observed between vitamin B12 and fT4 levels in our study indicates a potential link between vitamin B12 and thyroid hormones, necessitating further multicenter, comprehensive studies to clarify this relationship.

## Clinical Significance

In our study, it is recommended that thyroid dysfunction, which is less frequently observed in adolescents with vitamin B12 deficiency, should not be ignored.

## ETHICS APPROVAL

Ethics approval was obtained from the Academic Council of the University of Health Sciences Hamidiye Faculty of Medicine (Academic Council Decision No. 39, dated May 15, 2023; Ethics Approval No. 143, dated June 2, 2023).

## INFORMED CONSENT

Informed consent was waived by the ethics committee due to the retrospective nature of the study.

## CLINICAL TRIAL REGISTRATION

Not applicable. This was a retrospective observational study and not an interventional clinical trial.

## DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

## AUTHOR CONTRIBUTIONS

Conceptualization: Omer Guc, Raziye Dut; Methodology: Omer Guc, Raziye Dut; Data collection: Omer Guc; Formal analysis: Omer Guc, Raziye Dut; Writing – original draft: Omer Guc; Writing – review & editing: Raziye Dut; Supervision: Raziye Dut.

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Nil.

## AI USE DECLARATION

No artificial intelligence (AI) tools were used at any stage of this study or in the preparation of this manuscript.

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