Research Article

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A data analysis study: is there a relationship between 25(OH)D deficiency and iron-deficient anaemia in the pediatric population?

Bir veri analizi çalışması: Pediyatrik popülasyonda 25(OH)D eksikliği ile demir eksikliği anemisi arasında bir ilişki var mıdır?

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Abstract

Objectives: The purpose of this retrospective study was to investigate the relationship between 25 OH vitamin D (25[OH]D) deficiency and iron-deficient anaemia (IDA) in the pediatric population. This was aimed to provide a better insight to IDA follow-up and treatment.

Methods: The data of 120 patients diagnosed with IDA and 125 healthy pediatric patients were analyzed retrospectively. Serum vitamin B 12, Folate and 25(OH)D levels, between IDA and healthy groups were evaluated. The relationship between vitamins levels and IDA parameters were examined. Logistic regression analysis was used to assess whether 25(OH)D deficiency levels were an independent risk factor for diagnosing IDA.

Results: In the comparison of vitamins levels between groups, only mean serum 25(OH)D levels were found to be statistically significantly (p=0.000) lower (13.00 ± 2.50 ng/mL) in the group with IDA compared to the healthy group (25.98 ± 3.66 ng/mL). There were strong positive correlations between 25(OH)D deficiency levels and IDA. The deficiency of 25(OH)D levels was not found to be an independent risk factor for IDA (ORs: 0.958, 95% CI: 0.917–1.000).

Conclusions: Although current results confirm the association between 25(OH)D deficiency and IDA in pediatric patients, they indicate that there was no independent risk factor for IDA.

Keywords: Iron deficiency anemia; Vitamin B12; Folate; 25 OH vitamin D deficiency; Anemia panel tests of pediatric.

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Introduction

Iron deficiency (ID), an important public health problem, is among the most common nutritional deficiencies for developing countries. Iron deficiency anemia (IDA), is caused by inadequate iron availability for erythropoiesis. ID and IDA are frequently used interchangeably since ID is the most important cause of anemia all around the World [1, 2]. Iron deficiency is observed mostly in the pediatric age group, especially when the growth and the need for iron are predominant, as in the infantile and adolescence period [3]. Iron is required for hemoglobin (Hb) synthesis (hematopoiesis) in erythrocytes, and a low level of Hb leads to anemia. If ID is not prevented, it can lead to changes in the energy metabolism in the brain, neurotransmitter function and myelination defects. Therefore, babies and children with IDA are at risk of developmental challenges including cognitive, social-emotional, and adaptation problems [4–6].

Iron, vitamin B₁₂, and folate are required for basic metabolic functions. Deficiencies of these nutrients alone or in combination are common clinical conditions. Clinically, they have widespread effects not only with irregular hematopoiesis but also in other organs that may occur before the emergence of hematological abnormalities. The investigation of suspected ID, vitamin B₁₂ and folate deficiency should first aim at determining the presence of deficiency. It is essential to carefully evaluate the clinical symptoms and findings in order to direct the appropriate request and interpretation of the relevant laboratory tests. For an effective treatment for, it is necessary to determine the etiology of nutritional deficiency well. It is usually easy to correct the deficiency with supplements provided that compliance to treatment is ensured. Blood transfusion should be avoided if the level of Hb is not at the indication values for blood transfusion in patients unless otherwise is indicated by symptoms [7–11]. Furthermore, 25-hydroxyvitamin-D (25(OH)D) deficiency is a common condition. In the last decade, numerous “cross-sectional studies” which have indicated an association between low 25(OH)D levels and poor iron status and low erythrocyte values and transferrin saturation (TS) [12–14]. If some of these studies and suggested mechanisms are mentioned. For example, it is suggested that vitamin D may affect iron regulation and erythropoiesis by its influence on hepcidin via cytokines or independently of changes in pro-inflammatory markers [15, 16]. There are also findings which indicate direct influence of vitamin D on erythropoiesis precursors in bone marrow [13, 17]. Another part of the story is the evidence that a deficit in iron may disturb vitamin D synthesis (conversion of cholecalciferol to the biologically active form, calcitriol requires 2 hydroxylation steps—the first in the liver and the second in the kidney— which depend on enzymes containing heme, i.e., cytochromes P₄₅₀ [18].

Iron and 25(OH)D deficiencies are implicated with adverse health effects in children. Although many mechanisms have been explained as above, the potential relationship between the two is still not fully understood [19]. Therefore, 25(OH)D deficiencies is a matter of curiosity that is worth studying in the follow-up and treatment of IDA.

The purpose of this retrospective study was to investigate levels of vitamin B₁₂, folate and 25(OH)D deficiency in children diagnosed with Iron deficiency anemia (IDA). This was aimed to provide a better insight to IDA follow-up and treatment.

Material and methods

This retrospective study was conducted in accordance with the Declaration of Helsinki. Ethical approval (Date: 03.04.2020, No: 2020–07–124) was obtained from Kafkas University Faculty of Medicine, Local Ethics Committee.

Laboratory data of 362 pediatric cases, who admitted to Kafkas University Pediatric Outpatient Clinic between 2017–2019, were obtained from the hospital database and were evaluated retrospectively. Of these, 120 were pediatric patients (age range 1–15 years old) previously diagnosed with IDA by a pediatrician. For the diagnosis of IDA in pediatric patients, age-specific cut-off values of Hb, serum iron and ferritin (FER) levels were used as stated in the literature [20–22]. For the diagnosis of anemia, Hb cut-off level was accepted 11 g/dL for 6–59 months old, 11.5 g/dL for 5–11 years, 12 g/dL for 12–15 years old girls, 13 g/dL for 12–15 years old boys. Mean serum iron cut-off level was
accepted as $72 \pm 3.4$ (20–120) μg/dL for 2–6 years, $73 \pm 3.0$ (23–123) μg/dL for 6–12 years, $92 \pm 3.8$ (68–136) μg/dL for 12–18 years. In addition, FER cut-off level was accepted 12 ng/mL for under 5 years, 15 ng/mL for above 5 years.

The rest (n=125) of the cases were healthy children with no identified IDA but who had demographic characteristics similar to the patient group (1–15 years old and similar M/F gender ratio). These healthy cases had no acute and/or chronic infection, no malnutrition or obesity and no disease requiring chronic drug use. They were from the group of healthy children who were born in term and under routine growth and developmental follow-ups by Kafkas University Education and Research Hospital, Pediatric Outpatient Clinic between 2017 and 2019.

Pediatric patients who were born prematurely and had hypochromic microcytic anemia other than IDA and/or a diagnosis of thalassemia and/or malnutrition and/or syndromic findings, and/or drug use due to chronic disease (asthma, diabetes mellitus), and/or known hypoparathyroidism, hypoparathyroidism, hypoparathyroidism, hypoparathyroidism, and infection and inflammation were excluded from the study. In addition, in these two groups, those who had been prescribed vitamin D for the last 6 months or who had taken vitamin D supplements were not included in the study.

The demographic data, such as age and gender, of the patient and control groups, hematological parameters of erythrocyte count (RBC), Hb, Hematocrit (Hct) levels, mean erythrocyte volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), platelet count (PLT), cell distribution width of erythrocytes (RDW), and also, anemia panel tests of serum iron (SR), serum total iron-binding capacity (TIBC), FER, transferrin (Tf), TS, vitamin B12, and folate, and 25(OH)D levels were evaluated comparatively. The collection tubes Vacuette® (Greiner Bio-One GmbH, Germany) were used for serum analysis, with K2EDTA (BD Vacutainer®, BD Diagnostic, U.K.) for complete count and plasma analysis. Before analysis, all tubes were centrifuged at 3000 rpm for 5 min and supernatants were used to obtain serum and plasma samples.

In the study, the complete blood count analysis was performed using a flow cytometric method with an ABX-Pentra DX 120 (Horiba LTD, Japan) device, and serum biochemistry analysis was performed using the immuno-turbidimetric method with a Cobas C500 (Roche Diagnostik, Germany) device. Furthermore, vitamin B12, 25(OH)D, and folate levels were studied using the chemiluminescence immune enzymatic method with a UniCEL Dxl 800 (Beckman Coulter, USA) device (The analytical methods in the study were performed according to the procedures specified in the commercial kits suitable for each device). Performance of analytical methods were defined as: for vitamin B12, analytical sensitivity (minimum detection limit)=50 pg/mL, intra assay CV<4.8%, inter assay CV<6.6%; for folate, minimum detection limit=0.85 ng/mL, intra assay CV<2%, inter assay CV<3.4%; for 25(OH)D, minimum detection limit=2 ng/mL, intra assay CV<4.6%, inter assay CV<4.1%; for FER, minimum detection limit=0.2 ng/mL, intra assay CV<3.6%, inter assay CV<4.3%. Reference assay ranges of the analytical methods used are: Vitamin B12: 126.5–505 pg/mL, Folate: 3.1–19.9 ng/mL, 25(OH)D: 2–210 ng/mL. The cut-off level for diagnosis of 25(OH)D deficiency was defined as <20 ng/mL [23, 24].

Moreover, transferrin saturation (TS) in anemia panel was calculated using formula 1, and the Mentzer index was calculated using formula 2 [25, 26].

Formula 1: TS (%) = (Iron (μmol/L) / Transferrin (g/L) × 25.1) × 100
Formula 2: Mentzer index = MCV/RBC

SPSS 20.0 program was used for statistical analysis, and the conformity of variables to normal distribution was evaluated by the Kolmogorov–Smirnov test. It was determined that the numerical variables in two independent groups conformed to a normal distribution, and as a statistical analysis, Student’s t-test was applied to the variables to reveal the difference between the groups. Pearson correlation test was performed to determine the relationship between IDA parameters and vitamin B12, folate and 25(OH)D. In addition, the likelihood ratio of 25(OH)D deficiency levels as an independent risk factor for diagnosing IDA was demonstrated using binary logistic regression analysis (confidence intervals vs. Odds ratios [ORs] [95% CI]). For this purpose, the status of having an IDA diagnosis or not was consisted of as our dependent variable (the cut-offs of the parameters used for IDA diagnosis were defined above; Hb, FER, iron and IDA was 1, non-IDA was coded as 0), and our independent variable consisted of 25(OH)D levels. The values of p<0.05 were considered statistically significant in the interpretation of the results. The results are presented as mean ± standard deviation (SD) or min-max.

Results

The average age of a total of 120 patients (M/F: 63/57) in the patient group with IDA was 6.96 ± 5.16 years. The gender (M/F: 66/59) and average age (7.81 ± 4.90) distributions of 125 healthy individuals who constituted the control group were similar to the patient group. The changes in biochemical parameters between the groups were presented in Table 1. In pediatric patients with IDA, serum FER and TS decreased significantly; moreover, significantly lower 25(OH)D levels were shown compared to the control group.

The results of a complete blood count were presented in Table 2. While all hematological parameters were found to be lower in IDA group compared to the healthy control group, statistically significant increases were observed only in the RDW and Mentzer index values.

| Table 1: Means of biochemical parameters of the IDA and control groups. |
|---------------------------------|-----------------|-----------------|-----------------|
| **Biochemical analyses**        | **IDA (n:120)** | **Control (n:125)** | **p-Value**     |
| Serum ferritin, ng/mL           | 8.38 ± 2.08     | 41.31 ± 7.97     | 0.000           |
| Serum iron, μg/dL               | 19.14 ± 5.58    | 87.44 ± 16.21    | 0.000           |
| TIBC, μg/dL                     | 383.66 ± 48.80  | 282.56 ± 48.34   | 0.000           |
| Transferrin                     | 5.37 ± 2.13     | 32.04 ± 8.88     | 0.000           |
| saturation, %                   | 13.00 ± 2.50    | 25.98 ± 3.66     | 0.000           |
| 25(OH)D, ng/mL                  | 318.70 ± 108.23 | 345.31 ± 105.33  | 0.057           |
| Vitamin B12, pg/mL              | 13.04 ± 5.64    | 13.13 ± 5.37     | 0.395           |

p, indicates the significance in biochemical parameters between the groups according to Student’s t-test; SD, refers to standard deviation; IDA, refers to Iron deficiency anemia; TIBC, refers to the total iron-binding capacity; 25(OH)D, refers to 25-hydroxyvitamin-D levels.
Table 2: Means of hematological parameters of the IDA and control groups.

| Hematological analyses | IDA (n: n:120) Mean ± SD | Control (n:125) Mean ± SD | p-Value |
|------------------------|--------------------------|---------------------------|---------|
| RBC, ×10^12 L^-1       | 4.04 ± 0.35              | 5.54 ± 0.44               | 0.000   |
| Hb, g/dl               | 9.35 ± 0.63              | 13.24 ± 0.84              | 0.000   |
| Hct, %                 | 27.54 ± 2.21             | 40.96 ± 2.43              | 0.000   |
| MCV, fl                | 68.35 ± 2.68             | 82.11 ± 3.05              | 0.000   |
| MCH, pg                | 22.01 ± 2.06             | 31.58 ± 2.17              | 0.000   |
| MCHC, g/dl             | 27.54 ± 1.12             | 34.55 ± 1.34              | 0.000   |
| RDW, %                 | 17.68 ± 1.81             | 12.15 ± 0.92              | 0.000   |
| PLT, ×10^9 L^-1        | 339.43 ± 47.89           | 344.66 ± 71.52            | 0.499   |
| Mentzer index          | 17.51 ± 1.65             | 12.92 ± 1.43              | 0.000   |

p, indicates the significance in hematological parameters between the groups according to Student’s t-test; SD, refers to standard deviation; IDA, refers to Iron deficiency anemia; RBC, erythrocyte count; Hb, hemoglobin; Hct, hematocrit; MCV, mean erythrocyte volume; MCH, mean corpuscular Hb; MCHC, mean corpuscular Hb concentration; RDW, cell distribution width of erythrocytes; PLT, platelet count; Mentzer index, refers to MCV/RBC value.

When the relationship between IDA parameters and levels of vitamins is examined, there were only significant positive strong correlations between 25(OH)D and FER, iron, Hb, Hct and TS value (p=0.000, p=0.000, p=0.000, p=0.000 and p=0.000 with respectively; r=0.832, r=0.897, r=0.750, r=0.703 and r=0.786 with respectively). No significant correlation was found between other vitamins and IDA parameters (Table 3).

Table 3: Correlation between IDA parameters and 25(OH)D, B12 and folate.

| Correlations | FER | Iron | Hb | Hct | TS | Folate | 25(OH)D | Vitamin B12 |
|--------------|-----|------|----|-----|----|--------|---------|------------|
| FER Pearson correlation 1 | 0.833** | 0.678*** | 0.725** | 0.805** | 0.009 | 0.832** | 0.110 |
| Sig. (2-tailed) | 0.000 | 0.000 | 0.000 | 0.000 | 0.886 | 0.000 | 0.064 |
| Iron Pearson correlation 1 | 0.674** | 0.703** | 0.958** | -0.063 | 0.897** | 0.140 |
| Sig. (2-tailed) | 0.000 | 0.000 | 0.000 | 0.32 | 0.000 | 0.065 |
| Hb Pearson correlation 1 | 0.884** | 0.667** | -0.108 | 0.750** | 0.106 |
| Sig. (2-tailed) | 0.000 | 0.000 | 0.065 | 0.000 | 0.053 |
| Hct Pearson correlation 1 | 0.689** | 0.066 | 0.705** | 0.101 |
| Sig. (2-tailed) | 0.000 | 0.296 | 0.000 | 0.006 |
| TS Pearson correlation 1 | -0.095 | 0.786** | 0.119 |
| Sig. (2-tailed) | 0.13 | 0.000 | 0.057 |
| Folate Pearson correlation 1 | 0.084 | 0.019 |
| Sig. (2-tailed) | 0.182 | 0.762 |
| 25(OH)D Pearson correlation 1 | 0.105 |
| Sig. (2-tailed) | 0.052 |
| Vitamin B12 Pearson correlation 1 | 0.000 |

According to Pearson Correlation analysis: **Correlation is significant at the p<0.01 level (2-tailed). *Correlation is significant at the p<0.05 level (2-tailed). IDA, Iron deficiency anemia; FER, ferritin; Hb, hemoglobin; Hct, hematocrit; TS, transferrin saturation %; 25(OH)D, 25-hydroxyvitamin-D.

Table 4: Odds ratios (ORs) with confidence intervals (95% CI) of: 25(OH)D deficiency in diagnosis of IDA.

| 25(OH)D | ORs | 95% CI | p-Value |
|---------|-----|--------|---------|
| 25(OH)D | 0.958 | 0.917–1.000 | 0.062 |

Binary logistic regression analysis showing the effect between various criteria of IDA (Iron deficiency anemia) and 25(OH)D (25-hydroxyvitamin-D) deficiency levels: odds ratios (ORs) with confidence intervals (95% CI).

In addition, according to the result of the logistic regression model performed to determine an independent risk factor for IDA, deficiency of 25(OH)D levels which was shown to be associated with IDA, was not found to be an independent risk factor for IDA (ORs: 0.958, 95% CI: 0.917–1.000, p=0.062, Table 4).

Discussion

Anemia affects 2.5 billion people all around the world. According to the WHO data, it was reported that the prevalence of anemia was 47.4%, and the incidence of anemia increased in preschool children. Iron deficiency anemia (IDA) is responsible for approximately 50% of anemia cases in childhood [27, 28]. Vitamin D is an important steroid hormone for serum calcium and phosphorus metabolism and plays an important role in the function of various body systems. Pieces of evidence indicate that...
25(OH)D deficiency is associated with IDA. For example, it is observed that patients with IDA have fewer activities outside the home due to fatigue and malaise and are less exposed to sunlight. Therefore, it is predicted that 25(OH)D levels may also be low in this patient group. Furthermore, ID impairs the intestinal absorption of fat-soluble vitamins such as vitamin D [29–32].

In the study conducted by Monlezun et al. [33] the relationship between 25(OH)D levels and anemia was evaluated in 17-year-old individuals. They determined that the low vitamin D status was associated with the increased risk of anemia in the general population (25[OH]D<20 ng/mL). In another study, a significant relationship was found between IDA and vitamin D deficiency in children [28]. Hemoglobin and serum iron concentrations of children with a low concentration of 25(OH)D were found to be significantly low [33, 34].

In our study, the mean serum 25(OH)D levels (13.00 ± 2.50 ng/mL) were found to be significantly lower in pediatric patients with IDA compared to the control group (25.98 ± 3.66 ng/mL) (p=0.000). The results of other studies and our study revealed that patients with 25(OH)D deficiency are more likely to have IDA and low Hb levels [34, 35]. These findings were also supported by the correlation analysis conducted, it was a statistically significant strong positive correlation between 25(OH)D levels and the IDA parameters such as FER, iron, Hb, Hct, TS levels (Table 3). Therefore, since iron and vitamin D deficiencies are two common nutritional deficiencies and both nutrients interact with each other, it will be appropriate to monitor iron and vitamin D nutritional status simultaneously because, although some of the need for vitamin D is nutritionally provided in metabolism, none of the nutrients contain enough 25(OH)D to meet daily needs (20 ng/mL), and the most important source is vitamin D synthesized in the skin under the effect of sun exposure [23]. The effect of seasonal changes on serum 25(OH)D has been addressed in several previous studies. Studies have reported that it progresses at lower levels in the winter months compared to the summer months. In addition, although there are studies showing that the highest levels of 25(OH)D are between February-April and the lowest 25 (OH) D levels are between August and September [36], there are findings showing that they are at lower levels in winter and autumn compared to spring and summer [37]. Whatever happens, it is known that 25(OH)D levels change depending on the season. Geographical location and latitude affect the amount and quality of solar radiation, especially the intensity of UVB radiation reaching the earth. In regions with maximum latitude, the UVB does not have enough density to synthesize enough vitamin D in the fall or winter [37]. Therefore, the intake of sunlight should not be less, especially in winter and fall seasons. In this regard, in our study, we predicted that low mean serum 25(OH)D levels of children in both ID and the control group were caused by the fact that winter and fall seasons are long and harsh due to the geographical conditions (a latitude of 40.36°) of the region where we conducted the study, and that sun exposure was low due to negative effects of these seasonal conditions on children’s lifestyles (duration of stay in the house) and dressing styles. As a limitation of this study, it was not necessary to categorize 25(OH)D levels as seasonal due to these effects of latitude.

Vitamin D deficiency leads to an increase in the synthesis of hepcidin (either directly or indirectly by increase in pro-inflammatory cytokines). This hormone is synthesized in the liver and controls iron metabolism thus the absorption of iron from the intestinal epithelium decrease and its sequestration to macrophages increase. The restriction on the flow of iron into the erythropoietic bone marrow indirectly leads to a decrease in Hb synthesis [27, 38, 39]. This mechanism can explain why iron levels were found to be significantly lower in the 25(OH)D deficient group compared to the control group. Therefore, we believe that it could be more beneficial to evaluate vitamin D together with infection parameters to reveal its relationship with IDA and to clarify its therapeutic efficacy in improving anemia and that there may be a need for further multicenter clinical trials covering different geographical regions. We also suggest that a need for more comprehensive aiming to determine the role of vitamin D in iron metabolism and the panic values.

The folic acid nucleic acid is a water-soluble vitamin that plays a role in the synthesis of blood cells and nerve tissues. In its deficiency, it leads to megaloblastic anemia due to the prolonged synthesis phase of red blood cells. In addition to folic acid, vitamin B12 deficiency is the second common cause of megaloblastic anemia. Folic acid and vitamin B12 deficiencies impair DNA and folate synthesis, which causes impaired and ineffective erythropoiesis. In the deficiency of vitamin B12 and folate, tissues with rapid growth and rapid cell renewal are mostly affected. Therefore, problems related to these deficiencies are of great importance in childhood, during which the growth rate is high [30, 32, 40]. There is a limited number of studies in which the coexistence of folate and vitamin B12 deficiency was observed in children with IDA. Strikingly, in our study, no significant difference was found between the group with IDA and the control group in terms of mean folate levels. Furthermore, while the mean folate level of the groups was no significantly different in children with IDA and the control group in terms of mean folate levels. Strikingly, in our study, no significant difference was found between the group with IDA and the control group in terms of mean folate levels.
significantly lower and RDW and Metzner index were found to be higher in the group with IDA is the biggest indication that anemia due to ID in children is not macrocytic by highlighting that the group with anemia was selected correctly.

When the mean serum B12 vitamin levels between the group with IDA (318.70 ± 108.23 pg/mL) and the control group (345.31 ± 105.33 pg/mL) were compared, there was a decrease in vitamin B12 levels in the group with IDA. However, no statistically significant difference was observed. Furthermore, the fact that the mean serum B12 levels of the two groups were also within the normal reference range (126.5–505 pg/mL) was interpreted that there was no megaloblastic anemia associated with IDA in the study.

Ferritin (FER) is the name of the complex molecular family that binds iron. A FER molecule can bind up to 4,500 iron atoms. There is a strong correlation between serum FER and body storage iron levels. Nearly one-third of the body’s iron is bound to FER. Therefore, the changes in the body’s iron stores are reflected by the concentration of FER in the serum [41, 42]. The mean FER level (8.38 ± 2.08 ng/mL) was found to be lower in the IDA group compared to normal controls (41.31 ± 7.97 ng/mL). It was proven that low FER was certainly the most sensitive and most specific indicator of ID. A significant decrease was also observed in mean TS in children with IDA.

In this study, the 25(OH)D deficiency levels were questioned as to the possibility of being an independent risk factor for IDA. For this, binary logistic regression analysis was performed. Presence or absence of IDA was accepted as dependent variable. As a result, it was seen that deficiency of 25(OH)D levels in the diagnosis of IDA was not an independent risk factors (Table 4). However, some research suggests that 25(OH)D deficiency may be a risk factor for IDA [12]. Among the reasons why our finding was inconsistent with these studies may shown be that the ages of our sample are different from those in other studies, differences in our sample size and in the regression modeling used, and various difficulties arising from the retrospective nature of our study; For example, limitations such as the absence of other risk factors (such as nutrition, BMI, sun exposure, seasonal change and other clinical information) associated with 25(OH)D levels in the study planning, may be listed.

As a result of this study, although it was suggested that 25(OH)D deficiency in children may be associated with the etiopathogenesis of IDA, it was found that it was not an independent risk factor for IDA. In this context, more detailed studies are needed to explain the effect of 25(OH)D deficiency on IDA.

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