An investigation of vitamin B12, folate and vitamin D levels in pediatric patients with iron deficiency anemia

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research-article

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Demir eksikliği anemisi olan çocuklarda B_{12} vitamini, folat ve D vitamini düzeylerinin araştırılması

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Introduction

The metabolism of iron has a clinical significance in the human organism. Maintaining iron balance in the organism is basically regulated by a delicate balance between dietary iron intake and losses, and iron loss is kept at the minimum level possible [1]. Iron is lost from the body only through desquamation and bleeding. There is very little iron in the urine. Approximately 1 mg of iron is lost each day through desquamation. The menstrual loss is about 28 mg/cycle. Thus, if there is no bleeding in any other place, the iron loss is 1 mg/day in males and 2 mg/day in females of the fertility age. Apart from that, most of the iron required in the organism is provided by the reuse of existing iron. Approximately 1% of erythrocytes are phagocytosed by macrophages every day, and approximately 25–35 mg of iron into macrophages in this way[1]. Iron obtained from macrophages through the dietary iron intake is transported in the blood by transferrin (Tf) and is largely carried to the bone marrow. There is a relatively small amount of iron in the plasma Tf section. However, this iron constantly circulates and is renewed within a few hours. Excess iron is stored by parenchymal cells in the liver and reticuloendothelial macrophages. In cases that impair iron homeostasis, iron deficiency (ID) can be easily observed in humans [1, 2].

Iron deficiency (ID), an important public health problem, is among the most common nutritional deficiencies for developing countries. Since the most important indicator of ID is anemia, ID and IDA are frequently used interchangeably [2]. Iron deficiency anemia (IDA) is the most extensive cause of anemia all around the world and is caused by inadequate iron availability for erythropoiesis. Although iron deficiency (ID) is observed in the child age group, especially in growth periods, the infantile period and adolescence period, iron deficiency (ID) is frequently encountered due to the continuing growth and development in childhood and an increase in the need for iron, especially in certain age groups (periods with rapid growth, physiological loss in girls in adolescence) [3]. Iron is required for hemoglobin (Hb) synthesis (hematopoiesis) in erythrocytes, and a low level of Hb leads to anemia. If iron deficiency (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 hardship including cognitive, social-emotional, and adaptation problems [4-6].

Iron, vitamin B_{12}, 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 B_{12} 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 hemoglobin is not at the indication values for blood transfusion in patients unless otherwise is indicated by symptoms [7-11]. Furthermore, vitamin D deficiency is a common condition. Recent studies have shown that deficiency of 25-hydroxyvitamin-D (25-[OH]-D) is associated with the risk of anemia, which is a major public health problem. There are few studies exhibiting that is a relationship between the coexistence of vitamin D and ID and low serum 25-hydroxyvitamin-D (s-25-[OH]-D) and low serum iron (s-iron) levels, low erythrocyte values and transferrin saturation [12-14].

The purpose of this study was to appreciate the status of vitamin B12, folate and vitamin D levels in iron deficiency anemia (IDA) and to contribute to the literature by clarifying the underlying biochemical mechanisms.

Material and Methods

This study was conducted in accordance with the Declaration of Helsinki, and ethical approval dated 03.04.2020 was obtained from Kafkas University Faculty of Medicine Local Ethics Committee before starting the study (No: 2020-07-124). All laboratory data of 362 patients consisting of 192 pediatric patients aged between 1-15 years old who were diagnosed with iron deficiency anemia (IDA) by a specialist pediatrician, and 170 healthy children from among the children admitted to Kafkas University Education and Research Hospital Pediatric Clinic between 2017-2019, whose data were obtained completely, were taken from the hospital database and evaluated retrospectively. For the diagnosis of IDA in pediatric patients, age-specific cut-off values of hemoglobin (Hb) level, serum iron and ferritin levels were used as stated in the literature [15]. The data of pediatric patients, who were admitted to the routine pediatric polyclinic for growth and development follow-up, were born in term, had demographic characteristics similar to the patient group (1-15 age and M/F gender ratio), and had no acute and/or chronic infection, no malnutrition or obesity and no disease requiring chronic drug use, were used for the control group. In the study, the data of the patients who were born prematurely and had hypochromic microcytic anemia other than IDA, a diagnosis of thalassemia, malnutrition, syndromic findings, drug use due to chronic disease (asthma, diabetes mellitus), and infection and inflammation were excluded.

The demographic data, such as age and gender, of the patient and control groups, hematological parameters of erythrocyte count (RBC), hemoglobin (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 (SD), serum total iron-binding capacity (TIBC), ferritin (FER), transferrin (Tf), transferrin saturation (TS), vitamin B_{12} and folate levels, and vitamin D levels were evaluated comparatively.

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 B_{12}, vitamin D, and folate levels were studied using the chemiluminescence immune enzymatic method with a UniCEL DxI 800 (Beckman Coulter, USA) device. Moreover, transferrin saturation in anemia panel was calculated using formula 1, and the Mentzer index was calculated using formula 2 [16, 17].

Formula 1: Transferrin Saturation (%) = ([Iron (μmol/L)/ Transferrin (g/L) x 25.1] x 100)

Formula 2: Mentzer index=MCV/RBC

All pediatric patients admitted to Kafkas University Pediatric Clinic between 2017-2019 were retrospectively reviewed, and the data of 192 patients, who met the criteria of the study and whose laboratory data were obtained completely, and 170 healthy cases with similar demographic characteristics constituted the sample of the study.

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. 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 192 patients (M/F: 93/99) in the patient group with IDA was 7.0 ± 5.2 years. The gender (M/F: 82/88) and average age (7.6 ± 5.1) distributions of 170 healthy individuals who constituted the control group were similar to the patient group. The changes in biochemical parameters between the groups are presented in Table 1. In pediatric patients with IDA, serum ferritin and transferrin saturation decreased significantly; moreover, significantly lower vitamin D levels were shown compared to the control group.

The results of a complete blood count are 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.

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 [18, 19]. 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 vitamin 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 vitamin D levels may also be low in this patient group. Furthermore, iron deficiency (ID) impairs the intestinal absorption of fat-soluble vitamins such as vitamin D. Therefore, ID appears as an important factor in reducing serum vitamin D levels [20-23].

In the study conducted by Monlezun et al. [24] the relationship between vitamin 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 (vitamin D < 20 ng/mL). In another study, a significant relationship was found between IDA and vitamin D deficiency in children [25]. Hemoglobin and serum iron concentrations of children with a low concentration of vitamin D were found to be significantly low [24, 25].

In our study, the mean serum vitamin D levels (14.05 ± 2.05 ng/mL) were found to be significantly lower in pediatric patients with IDA compared to the control group (26.45 ± 4.95 ng/mL) ($p < 0.001$). The results of other studies and our study revealed that patients with vitamin D deficiency are more likely to have IDA and low hemoglobin levels. 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 vitamin 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. Therefore, the intake of sunlight should not be less, especially in winter and fall seasons. In this regard, we predicted that both ID and low mean serum vitamin D levels of children in the control group were caused by the fact that winter and fall seasons are long and harsh due to the geographical conditions 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 [26]. Vitamin D deficiency increases the synthesis of hepcidin, which is a hormone that controls iron metabolism and is synthesized in the liver, by increasing pro-inflammatory cytokines, and therefore, the absorption of iron from the intestinal epithelium and its release from macrophages decrease, so it may also indirectly lead to a decrease in hemoglobin synthesis [18, 27]. This mechanism can explain why vitamin D levels were found to be significantly lower in the group with IDA compared to the control group, which is another result of our study. 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.

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 B_{12} deficiency is the second common cause of megaloblastic anemia. Folic acid and vitamin B_{12} deficiencies impair DNA and folate synthesis, which causes impaired and ineffective erythropoiesis. It is very important that vitamin B_{12} and folate levels are normal in the development stage of many organ systems such as the central nervous system, cardiovascular system, and hematopoietic system, especially in children. In the deficiency of vitamin B_{12} 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 [21, 23, 28]. There is a limited number of studies in which the coexistence of folate and vitamin B_{12} 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 observed to be within normal limits (4.6-18.7 ng/mL), the fact that MCV and MCH levels were found to be statistically 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 B_{12} vitamin levels between the group with IDA (258.1 ± 59.9 pg/mL) and the control group (295.2 ± 58.7 pg/mL) were compared, there was a decrease in vitamin B_{12} levels in the group with IDA. However, no statistically significant difference was observed. Furthermore, the fact that the mean serum B_{12} levels of the two groups were also within the normal reference range (160-800 pg/mL) was interpreted that there was no megaloblastic anemia associated with IDA in the study.

Ferritin is the name of the complex molecular family that binds iron. A ferritin molecule can bind up to 4.500 iron atoms. There is a strong correlation between serum ferritin and body storage iron levels. Nearly one-third of the body's iron is bound to ferritin. Therefore, the changes in the body's iron stores are reflected by the concentration of ferritin in the serum [29, 30]. The mean ferritin level (10.7 ± 5.1 ng/mL) was found to be lower in the IDA group compared to normal controls (51.8 ± 12.3 ng/mL). It was proven that low ferritin was certainly the most sensitive and most specific indicator of ID. A significant decrease was also observed in mean transferrin saturation in children with IDA.

Vitamin D appears to be a biomarker that may be a candidate to be included in routine practice in addition to hemogram and anemia panel in the clinical diagnosis and monitoring of IDA. There is a need for more comprehensive multicenter studies aiming to determine the role of vitamin D in iron metabolism and the panic values.

Conflict of Interests Statement: The authors report no conflicts of interest.

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