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Evaluation of serum vitamin D levels in premenopausal women with iron deficiency anemia

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ABSTRACT

Objectives: In recent years, many effects of vitamin D except on bone metabolism have been discovered. Vitamin D contributes to the correction of the anemia by acting on the erythroid precursors in the bone marrow via Vitamin D Receptor and provides the elimination of free radicals and prooxidant substances secondary to iron deficiency due to its antioxidant effect in iron deficiency anemia (IDA).

Methods: A total of 97 female premenopausal women aged 18-44 were included in the study. Fifty patients with hemoglobin levels below 12 mg/dl and iron deficiency were classified as IDA group, and 47 subjects with hemoglobin levels of 12 mg/dl and above were classified as control group. The demographic data and biochemical parameters of all patients included in the study were analyzed.

Results: The vitamin D of the patient group was found to be 7.87 ± 3.63 ng/ml and the vitamin D of the control group was 11.84 ± 6.72 ng/ml. The difference between the groups was statistically significant. There was a positive correlation between serum vitamin D and serum hemoglobin, hematocrit, serum MCH, serum iron level, transferrin saturation index, ferritin.

Conclusions: In the light of the results of our study and other studies in the literature, we think that vitamin D deficiency may be important in patients with IDA and that vitamin D deficiency in these individuals will contribute to the regulation of anemia due to positive effects of vitamin D on both erythropoiesis and hepcidin in IDA are considered. However, larger studies are needed to clarify this issue.

Keywords: Vitamin D, iron deficiency anemia, anemia

Iron, is a vital element and essential for erythropoiesis, oxidative metabolism and cellular immune response. It has important functions in oxygen transport, catalysis of many enzymes in the energy system and the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein [1, 2]. As a result of cell metabolism, free radicals and reactive oxygen derivatives are formed. Free radicals show their effects on the cell membrane, organelles and DNA by causing protein, lipid, carbohydrate and DNA oxidation. These free radicals and reactive oxygen derivatives are neutralized by a complex antioxidant system [3]. It is accepted that oxidative stress increases in iron deficiency anemia (IDA) due to both increased oxidant amount and decreased antioxidant enzyme capacity [4]. The production of iron-containing proteins such as cytochrome, myoglobin, catalase and peroxidase is also affected in iron deficiency [5]. It has been shown in

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many studies that the activity of enzymes that protect cells against oxidative damage is impaired and thus tissues are exposed to oxidative stress in IDA [2].

In recent years, the determination of vitamin D receptor (VDR) in many tissues has revealed new opinions about the function of this vitamin. Many studies have shown the role of vitamin D deficiency in the formation of autoimmune diseases, inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, diabetes, infectious diseases, many types of cancer and heart diseases [6-8]. It was also determined modulator, antiinflammatory, antioxidant, antidiabetic, antihypertensive and renoprotective effectsof vitamine D [9]. Studies have shown that active vitamin D decreases proliferation and production of immunoglobulin [10] and affects erythropoiesis [11, 12]. Vitamin D levels are hundreds of times higher in the bone marrow compared to plasma and have been shown to be effective in regulating the functions of bone marrow. Red blood cells are prevented from becoming active in vitamin D deficiency [13]. It was also found to directly stimulate erythroid precursors [14]. Vitamin D, due to it's antioxidant and antiinflammatory effect, eliminate free radicals and prooxidant substances as well as through the VDR in the bone marrow contribute to the correction of anemia by acting on erythroid precursors. The aim of this study was to evaluate the effect of vitamin D levels on IDA in premenopausal women.

METHODS

A total of 97 female premenopausal women aged 18-44 were included in the study. Fifty patients with hemoglobin levels below 12 mg/dl and iron deficiency were classified as IDA group, and 47 subjects with hemoglobin levels of 12 mg/dl and above were classified as control group. Ethics Committee approval was obtained from the Ethics Committee of our center on 11.11.2015 and numbered 136. Exclusion criteria in our study; (i) detection of vitamin B12 or folic acid deficiency along with IDA, (ii) usage any iron preparation in the last 6 months before the study, (iii) presence of active infection, rheumatoid arthritis, ankylosing spondylitis, collagen tissue disease, celiac, hypo-hyperthyroidism, hypo-hyperparathyroidism, (iv) usage preparations such as calcium and vitamin D, bisphosphonates, calcitonin, selective estrogen receptor modulators, immunosuppressive drugs, antiepileptics, steroid, (v) presence of bone diseases, cushing syndrome, liver and kidney disease presence of malignancy, malnutrition and malabsorption. In the selection of individuals in the control group; the absence of any additional disease and the absence of IDAwas taken as the criterion. The demographic data and biochemical parameters of all patients included in the study were analyzed.

Complete blood counts were taken to tubes containing ethylenediaminetetraacetic acid (EDTA) and analyzed with Bt pro 2401. Serum AST, ALT, ALP, urea, creatinine, calcium, phosphorus, iron, total iron Binding Capacity (TIBC) levels were determined by the biochemistry analyzer Cobas 6000 C501 (Roche Diagnostics GmbH, Mannheim, Germany). Serum 25-Hydroxyvitamin D (25(OH)D), parathormone, vitamin B12, folic acid, ferritin levels were determined by Cobas e 411 (Roche Diagnostics GmbH, Mannheim, Germany).Body Mass Index (BMI); was calculated by dividing the weight of the patient by the square of his/her height (kg/m^2) by using Quetlet index.

Statistical Analysis

Statistical analyzes were performed using SPSS 22.0 program (SPSS Inc. Chicago, IL).The normal distribution of the variables was tested by Kolmogorov smirnov and the variance equation was tested with the Levene test. Because of the normal distribution of the data, all analyzes were performed using parametric tests. Continuous variables were expressed as mean ± standard deviation and categorical variables as percentage. Independent-Samples t-test was used for numerical variables and chi-square test was used for categorical ones. The relationship between vitamin D and other laboratory values was evaluated by Pearson correlation analysis. In the paired comparisons, the parameters which were statistically significant were included in the multivariate model. Stepwise logistic regression analysis was used to determine the independent risk factors of IDA. A receiver operator characteristic (ROC) curve was used to determine the cut-off value of vitamin D level for IDA. The area under the curve (AUC) was calculated for the accuracy of the test. A $p < 0.05$ was considered significant in all comparisons.

Table 1. Demographic and laboratory datas of the groups

Variables	PatientGroup (n: 50)	Control Group (n: 47)	*p* value
Age (years)	28.80 ± 8.06	28.27 ± 6.53	0.727
BMI (kg/m²)	23.83 ± 3.82	23.64 ± 3.12	0.796
Hg (gr/dl)	10.39 ± 1.09	14.06 ± 0.65	**< 0.001**
Hct (%)	30.63 ± 3.14	41.33 ± 2.13	**< 0,001**
Iron (µg/dl)	28.22 ± 8.98	104.02 ± 30.8	**< 0.001**
TIBC	433.28 ± 59.09	352.43 ± 52.61	**< 0.001**
TSI (%)	6.591 ± 2.32	29.98 ± 9.61	**< 0,001**
Ferritin (ng/ml)	5.95 ± 2.87	36.88 ± 17.93	**< 0.001**
Vitamin D (ng/ml)	7.87 ± 3.63	11.84 ± 6.72	**0.01**

BMI = Body mass index, TSI = Transferrin saturation index, TIBC = Total iron binding capacity

RESULTS

The mean age of the patients included in our study was 28.80 ± 8.06 years in the patient group and 28.27 ± 6.33 years in the control group. There was no statistically significant difference between the groups (p = 0.727) (Table 1). In the statistical analysis of vitamin D levels, the vitamin D level of the patient group was found to be 7.87 ± 3.63 ng/ml and the vitamin D level of the control group was 11.84 ± 6.72 ng/ml. The difference between the groups was statistically significant (p = 0.01) (Fig. 1) (see Table 1).

In the statistical analysis, there was independent correlation between vitamin D level and IDA in logistic regression analysis (B = 1.168, p = 0.02). The cut-off value of vitamin D level 9.12 was 74% sensitive and 40% specific for IDA. In the ROC curve analysis, the AUC value was 0.681 (95% CI = 0.575-0.778, p < 0.02) (Fig. 2).

In our study, the correlation between serum vitamin D level and hemogram and iron parameters was found; There was a positive correlation between serum vitamin D level and serum hemoglobin level (r = 0.393, p < 0.001) between serum vitamin D level and serum hematocrit level (r = 0.419, p < 0.001). And

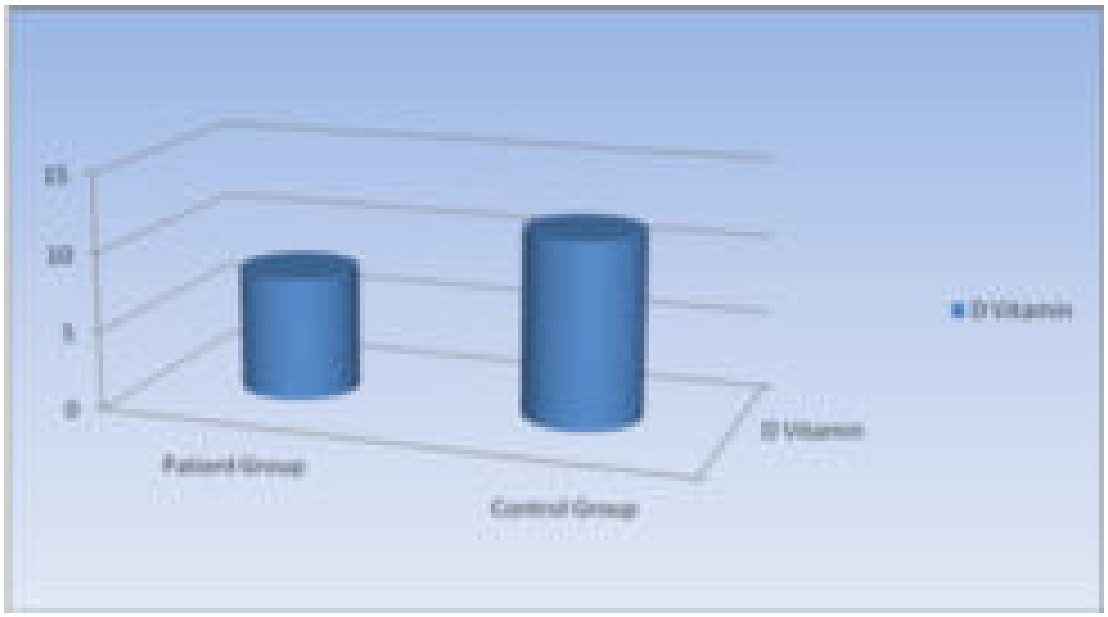

Fig. 1. Vitamin D levels of the groups.

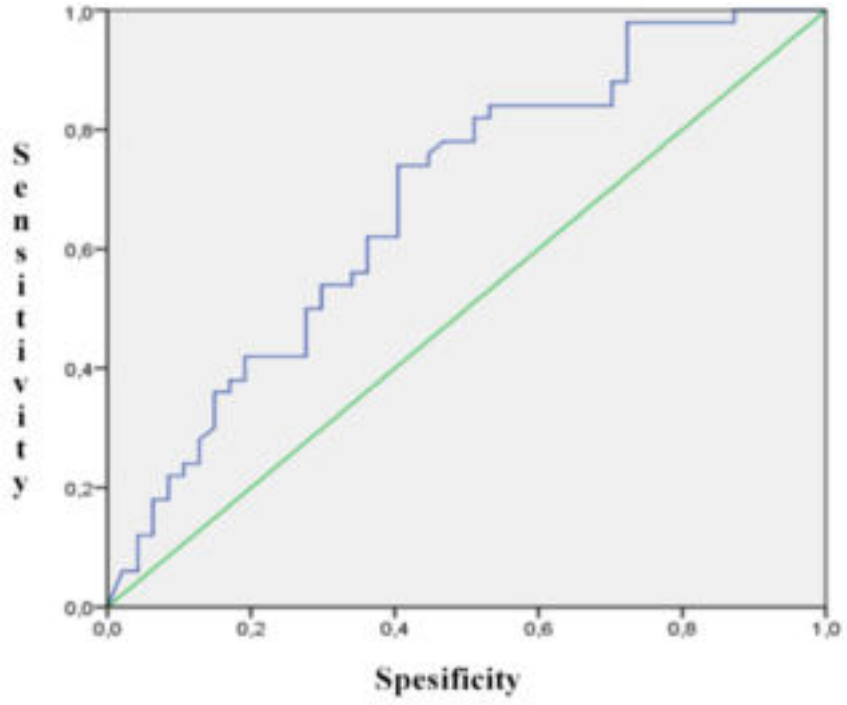

Fig. 2. Logistic regression graph of iron deficiency anemia parameters with vitamin D.

also positive correlation was found between serum vitamin D and serum MCH level (r = 0.298, *p* = 0.003), serum iron level (r = 0.301, p = 0.003), TSI (r = 0.249, *p* = 0,014), serum ferritin level (r = 0.225, *p* = 0.026). But there was a negative correlation between serum vitamin D level and serum RDW level (r = -0,225, *p* = 0.027).

DISCUSSION

Iron is an essential trace element for erythropoiesis, oxidative metabolism and cellular immune response, which has an effect on many systems [2, 15]. In many studies, it has been suggested that IDA has effects on cellular functions, growth, motor and mental development, behavior and cognitive functions, immune system, gastrointestinal system and physical capacity [16]. The main task of erythrocytes is to carry oxygen. Erythrocytes, where oxidative events occur at any time, because they are constantly exposed to oxygen, are equipped with an extremely effective antioxidant defense system.

Unlike other cell types, there are many active antioxidant enzymes such as super oxide dismutase (SOD) and catalase. Decreased enzymatic antioxidant capacity of erythrocytes in IDA has been reported [17-19]. Oxidative stress caused by the decrease in antioxidant enzyme activities in erythrocyte negatively affects oxidant/antioxidant system in serum. Serum antioxidant capacity cannot improve the increased oxidative state and result in increased oxidative stress [20]. In the study performed by Yoo *et al*. [21], oxidative capacity was significantly higher in the IDA group compared with the control group, and total antioxidant and catalase activity were found to be low. After four months of treatment, oxidant, antioxidant and catalase activity were found to be similar with control group [21].

Vitamin D has an immunomodulatory, anti-inflammatory, antioxidant, antidiabetic, antihypertensive and renoprotective positive effects [9]. Active vitamin D has been reported to reduce the production of many inflammatory cytokines (IL-2, IL-6, IL-12, IFN-tı, TNF-α, TNF-ß), [22], to cause cellular proliferation and to reduce the production of immunoglobulin [23].

Decreases in 25(OH)D levels may suppress erythropoiesis in bone marrow by decreasing local calcitriol production. Calcitriol has a direct proliferative effect on erythroid series cells. Endogenous erythropoietin and calcitriol have a

synergistic effect. In addition, calcitriol upregulate erythropoietin receptors in erythroid progenitor cells [24, 25]. In addition, calcitriol has a key role on the immune system and has an inhibitory effect on proinflammatory cytokine expression.Vitamin D is thought to be an inhibitory effect of anemia by its inhibitory effect on specific inflammatory pathways [26]. In the study conducted by Sim *et al*. [27], the probability of developing anemia was 1.86 times higher in people living in the south of the USA with vitamin D deficiency (< 30 ng/ml). But there is no difference according to gender [27]. A total of 2,526 people were included in the study to investigate the relationship between vitamin D deficiency and IDA in Korean girls and boys and adolescents. It was found that the socioeconomic level of patients with IDA was lower, BMI was higher and vitamin D level was lower.As vitamin D levels increased, it was observed that IDA was less frequent but this difference was not found in men [28]. In another study, 158 pregnant women were included in the study. Vitamin D and iron levels were measured at the 25th and 40th weeks of gestation and the risk of IDA was found to be eight times higher in patients with vitamin D levels below 50 nmol/l [29]. Anemia that occurs in patients with vitamin D deficiency was previously attributed to the deficiency of erythropoietin production [30, 31]. However, recent studies also emphasize the role of hepcidin, a hepatic peptide [32]. Hepcidin is a systemic iron-regulating hormone. High plasma hepcidin levels lead to iron sequestration in macrophages, contributing to the pathogenesis of anemia by restricting the flow of iron into the erythropoietic bone marrow.Vitamin D deficiency was found to cause hepcidin upregulation [33].

In our study, the vitamin D levels were 7.87 ± 3.63 ng/ml and 11.84 ± 6.72 ng/ml in the IDA group and control group, respectively. There was a significant difference between the groups (p = 0.01). However, vitamin D level of both groups is deficient. Vitamin D deficiency in most of the participants shows that vitamin D deficiency is a public health problem in our country which should be taken seriously. One of the factors affecting the level of vitamin D is the degree of utilization of sunlight according to the latitude and longitude of the geographic region and also one of the personal factors affecting the vitamin D level is the style of clothing. Clothes constitute an important barrier between UV heat and skin [34]. The fact that most of the participants in our study have a closed clothing style may be another reason why vitamin D levels are so low.

Limitations

Our study has some restrictive aspects. In this cross-sectional, retrospective study, it is not possible to determine the exact pathophysiology of the relationship between vitamin D and IDA.The fact that hepcidin levels were not measured limit the explanation of the pathogenesis of anemia in patients.

CONCLUSION

In our study, although the serum vitamin D level of the patient group and the control group was below the reference value (< 20 ng/ml), the difference between the groups was statistically significant (p = 0.01). There was a positive correlation between serum vitamin D level and serum hemoglobin, hematocrit, MCV, MCH, iron, transferrin saturation index, ferritin level, and negative correlation between serum RDW levels.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The authors disclosed that they did not receive any grant during conduction or writing of this study.

Authors' Contribution

All of the authors have contributed to the study on conception and design, drafting the article, revising it critically for important intellectual content, and final approval of the version to be published. All authors are in agreement with the content of the manuscript.

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