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The effect of vitamin D3 deficiency on hematological indices in Isfahan patients

Seyedmahdi Tabatabaei[1,2], Fatemeh Kianinodeh[3], Atena Alibakhshi[4], Sahar Hormozi[5], Somayeh Saadi[6], Omid Nikpayam[7]

[1]*Department of Histology and Embryology, Faculty of Medicine, Pamukkale University, Denizli, Türkiye.*
[2]*Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran*
[3]*Endocrine Research Center, Institue of Endocrinology and Metabolism, Iran University of Medical Science, Tehran, Iran.*
[4]*Department of Laboratory Sciences, Borujerd Branch, Islamic Azad University, Borujerd, Iran*
[5]*Department of Clinical Research, Pasteur Institute of Iran, Tehran, Iran.*
[6]*Department of Biochemistry, Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.*
[7]*Department of Clinical Nutrition and Dietetics, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

ABSTRACT

Aim: Since suboptimal vitamin D3 levels may be associated with anemia and several disorders, the study presented here investigated hematological indices state at patients with vitamin D3 deficiency.

Methods: This cross-sectional study performed in Isfahan, Iran between 2017 and 2019. Initially, patient vitamin D3 level was determined. Based on vitamin D3 reference ranges them were divided into two vitamin D3-deficient (vitamin D3<25ng/mL) and control groups (>25ng/mL). Completed blood count was measured by hematology analyzer. Alkaline phosphatase (ALK) level was also measured and the results were statistically analyzed.

Results: Of 1925 studied patients, 621 (32.3%) patients were male and 1274 (67.7%) females. 909 patients have an abnormal levels of vitamin D3 (<25ng/mL) and 1016 patients have sufficient vitamin D3 level. A weak correlation observed between white cell count and mean cell volume and low level of vitamin D3 (beta = 0.11). We also found a significant difference in mean age of patients with vitamin D3-deficient compared to control (p = 0.003). Moreover, we found no significant change in serum ALK levels between patients with vitamin D3 deficiency and control as well as subgroups of vitamin D3 deficiency.

Conclusions: Vitamin D3 deficiency may not have significant impact on red cell count and indices, differential white blood cell count, and platelet count. It is suggested that a prospective cohort study be conducted in the future.

Key words: 1,25-(OH)2D3, mean cell hemoglobin, mean cell hemoglobin concentration, platelet, white blood cell.

✉ *Fatemeh Kianinodeh,*
Endocrine Research center, Institue of Endocrinology and Metabolism, Iran University of Medical Science, Tehran, Iran
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Introduction

25-hydroxyvitamin D (25[OH]D) is an important steroid hormone involved in wide range of physiological processes including calcium and phosphate metabolism, cellular

differentiation and growth in bone marrow, and erythropoiesis [1-3]. Vitamin D3 mediates its function by binding to the vitamin D receptor (VDR) in various tissues such as bone marrow, erythroid precursors, and stromal cells [3, 4]. It is seem that active vitamin D3 directly stimulates erythropoiesis through proliferating erythroid precursors including burst-forming unit-erythroid (BFU-E) [5-7]. A long with this observation, several lines of evidence indicates that insufficient levels of vitamin D3 is associated with anemia, decreased levels of hemoglobin, and increased usage of erythrocyte-stimulating agents [2,7,8]. Icardi et al also demonstrated that vitamin D administration and analogues improved anemia and reduced erythropoietin (EPO) requirements [9]. Moreover, in vitro studies demonstrated that vitamin D3 has an anti-inflammatory function and declines the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-1β (IL-1β), suppressing the mRNA expression of hepcidin antimicrobial peptide (HAMP), which is account for the iron hemostasis [9-12]. Downregulation of HAMP expression leads to increased ferroportin mRNA expression, a cellular iron exporter, yielding sufficient iron available for hemoglobin synthesis and erythrocyte formation [10,13]. Vitamin D deficiency is also associated with an increased risk of heart failure, hypertension, and some autoimmune disorders including systemic lupus erythematosus (SLE), type 1 diabetes mellitus (T1DM), and rheumatoid arthritis [3, 14-18]. Given these findings, maintaining sufficient Vitamin D3 level is necessary for regulating normal biologic actions and anemia prevention and is essential to rule out its deficiency [1, 4, 11]. Regarding key function of vitamin D in the red blood cell formation, the current study investigated the effect of vitamin D deficiency on hematological indices. Furthermore, considering the measurement of serum alkaline phosphatase activity is used as a routine biochemistry test for recognizing and screening of hypovitaminosis D [19], its level was also measured in this study.

Materials and metods

This cross-sectional study was done in Isfahan, Iran between 2017 and 2019. A total of 1925 patients were included in this study 621 (32.3%) patients were male and 1274 (67.7%) females. Subjects 35-45 years old and patients with serum vitamin D3 levels <25 ng/ml were included, while children, patients with chronic liver disease, and taking any drugs that could affect the liver or bone metabolism were excluded in this study.

Hematological analysis, including hemoglobin concentration (Hb), hematocrit (HCT), red cell indices (including, mean cell volume [MCV], mean cell hemoglobin [MCH], mean cell hemoglobin concentration [MCHC], red cell distribution width [RDW]), white blood cell (WBC) count, differentiation WBC count, platelet count, and platelet distribution width (PDW) were measured using a hematology full blood analyzer (Sysmex KX-21N hematology analyzer). Biochemical assay, including Serum alkaline phosphatase (ALK) levels was measured by colorimetric methods using a Chemistry Analyzer (Mindray BS3000, china). The samples were incubated in the appropriate reaction mixture (10 mmol/L P-Nitrophenyl phosphate [PNPP], 1 mmol/L diethanolamine [DEA], and 0.5 mmol/L magnesium chloride [MgCl2]) for 1 min at room temperature, and the absorbance was evaluated at 405 nm. Standard curve was applied to assess the values of this parameter. Serum vitamin D (25-hydroxycholecalciferol) was also measured in serum patients by competitive ELISA- based immunoassay that competes for

monoclonal antibodies coated to microplate wells between 25-OH vitamin D3 of serum patient and 25-OH vitamin D3-Biotin. Finally, the optical absorption of the samples obtained at 450 nm. In this study, patients with a vitamin D3 level < 25 ng/ml was considered as vitamin D3-deficient and patients with >25 ng/mL vitamin D3 were introduced as control groups. Furthermore, patients were divided into groups based on decreased vitamin D3 levels; low and intermediate vitamin D3 levels. 161 (17.7%) patients have less than 10 ng/mL vitamin D3 while 768 (82.3%) patients have intermediate vitamin D3, i.e., 10-24 ng/mL.

Statistical analysis was performed using SPSS (Statistical Software for Social analysis-version 13) by a statistician and using linear regression analysis. A *p*-value < 0.05 was considered statistically significant.

Results

The correlation between vitamin D3 deficiency and age, gender, and hematological indices

A total of 1925 patients were included in this study 621 (32.3%) patients were male and 1274 (67.7%) females. Of 1925 studied patients, 909 patients have a lower levels of vitamin D3 and 1016 patients have sufficient levels of vitamin D3 and considered as control group. As presented in table 1, mean age of patients with abnormal levels of vitamin D3 was significantly less than control group (p = 0.003) with no significant changes in hematological parameters between patients with vitamin D3 deficiency and control. No significant different in age, gender, and hematological parameters observed among of sub-groups. 51.3% of female patients had an

Table 1. The changes in demographic, hematological indices, and ALK levels in patients with vitamin D3 deficient and control groups.

Parameters	Abnormal level of Vitamin D3 (Patients, n=909)	Sufficient level of Vitamin D3 (Control, n=1,016)	*P* value
Male	301 (36.4%)	320 (34.1%)	0.009
Female	578 (63.5%)	696 (68.5%)	0.001
Age (years)	38.6	40.7	0.003
WBC count ($\times 10^9$/L)	6.89	6.5	0.239
RBC count ($\times 10^9$/L)	5.30	4.67	0.861
Hb (g/dL)	14.1	14.2	0.298
HCT (%)	41.5	41.9	0.243
MCV (fL)	84.4	84.6	0.775
MCH (pg)	28.89	28.80	0.696
MCHC	33.7	33.6	0.719
Platelet count ($\times 10^9$/L)	237	233	0.091
PDW	12.1	11.7	0.305
RDW	13.2	12.9	0.732
% Neutrophil	60.2	63	0.887
% Basophil	0.43	0.73	0.901
% Lymphocyte	36.9	35.4	0.171
% Eosinophil	2.2	2.1	0.146
ALK (IU/L)	203.6	201.4	0.564

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, HCT: hematocrit, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration, PDW: platelet distribution width, RDW: red cell distribution width, and ALK: alkaline phosphatase, fL: femtoliter, g/dL: gram/deciliter, IU/L: International unit/liter.

Table 2. The changes in demographic, hematological indices, and ALK levels in subgroups of vitamin D3 deficiency.

Parameters	Sub-grouped patients		
	Low level of Vitamin D3 (< 10 ng/ml)	**Intermediate level of vitamin D3 (10-24 ng/ml)**	***P* value**
Male	50 (5.5%)	281 (30.9%)	0.112
Female	111 (12.2%)	467 (51.3%)	0.23
Age (years)	38.9	38.4	0.831
WBC count ($\times10^9$/L)	6.32	7.39	0.398
RBC count ($\times10^9$/L)	4.98	5.63	0.645
Hb (g/dL)	14.2	14.1	0.793
HCT (%)	41.7	41.3	0.672
MCV (fL)	84.4	84.5	0.974
MCH (pg)	28.89	28.86	0.562
MCHC	33.7	33.6	0.8
Platelet count ($\times10^9$/L)	235	239	0.532
PDW	11.3	12.9	0.586
RDW	13.5	12.9	0.510
% Neutrophil	56.1	64.3	0.620
% Basophil	0	0.86	0.638
% Lymphocyte	37.2	36.6	0.623
% Eosinophil	2.2	2.2	0.675
ALK (IU/L)	201.6	205.6	0.761

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, HCT: hematocrit, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration, PDW: platelet distribution width, RDW: red cell distribution width, and ALK: alkaline phosphatase, fL: femtoliter, g/dL: gram/deciliter, IU/L: International unit/liter.

Table 3. The relationship between vitamin D3 deficiency and hematological indices.

Vitamin D	**Coef.**	**Std. Err.**	**t**	***p*> | t |**	**[95% conf.**	**Interval]**
Age	-.003666	.0036421	-1.01	0.317	-.0104766	.00355589
Sex	-7.308254	6.746921	-1.08	0.283	-20.72804	6.111529
WBC count	0.119138	.051911	2.30	0.024	.0161604	.2221155
RBC count	-.0109418	.0168272	-0.65	0.517	-.0443224	.0224388
HB	.0568992	.066069	0.86	0.391	-.074164	.1879624
HCT	-.00797754	.0322813	-0.25	0.805	-.0720127	.056062
MCV	.1424968	.0606989	2.35	0.021	.0220866	.2629071
MCH	-.3579457	.1731359	2.08	0.040	.0141419	.5845306
MCHC	.2993362	.14037667	1.84	0.068	-.0192106	.5366314
PLT	-.0022028	.0014511	-1.52	0.132	-.0050814	.0006757
RDW	.0550904	.0850883	0.65	0.519	-.1137019	.2238827
MPV	-.0753419	.0667496	-1.13	0.262	-.2077552	.0570713
PDW	-.0086859	.007127	-1.22	0.226	-.0228239	.0054521
Neu	-.0262529	.0377973	-0.69	0.489	-.1012326	.0487268
Lym	-.021032	.0387789	-0.54	0.589	-.0979589	.0558948
Eos	-.1048642	.069745	-1.50	0.136	-.2432197	0.0334912
ALP	.0001522	.0004904	0.31	0.757	-0.0008206	.001125

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, HCT: hematocrit, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration, PDW: platelet distribution width, RDW: red cell distribution width, and ALK: alkaline phosphatase, fL: femtoliter, g/dL: gram/deciliter, IU/L: International unit/liter.

intermediate level of vitamin D3, while 30.9% of males showed it. In the subgroup with low level of vitamin D3, 12.2% were females and 5.5% were males. In addition, there was no significant different between subgroups (table 2). As shown table 3, with the exception of the MCV and WBC count, there was a reverse correlation between other hematological parameters (RBC count, MCH, MCHC, RDW, Plt count, PDW, Neu, Lym, Mono, and Eos) and vitamin D levels, although this correlation was weak. This means that followed by increment vitamin D3 levels, the aforementioned hematological indices levels decreased. We also found a direct weak correlation between abnormal level of vitamin D3 and WBC count and MCV parameters (Coef. = 0.11), so that the increase of these two parameters is affected by the abnormal levels of vitamin D3 (table 3).

The correlation between vitamin D3 deficiency and serum ALK levels

There is also no significant changes in serum ALK levels between patients with abnormal levels of vitamin D3 and control as well as sup-groups of vitamin D3 deficiency (tables 1 and 2).

Discussion

The present study investigated the effect of vitamin D3 deficiency on the hematological indices. For this purpose, the serum vitamin D3 level and complete blood cell count of 1925 patients were measured. Serum ALK levels also determined. Patients were divided into vitamin D3 deficient (<25 ng/mL) and control groups (>25 ng/ml) based on vitamin D3 level. Our results showed no significant different in hematological indices between patients with vitamin D3 deficiency and control groups. This may be due to the presence of some confounders in this study. One of these important factors is gender (male and female), which is not equally distributed in the two groups, and as mentioned above, of 1925 patients, 68.5% were female and 36.4% were male. A probable reason can be due to sufficient sunlight exposure or ingestion of vitamin D supplements in the Isfahan populations. We found no data about the vitamin D supplement ingestion of patients. We detected a weak correlation between white blood cell count and MCV and low level of vitamin D3. We also found a significant difference in mean age of patients with abnormal levels of vitamin D3 than control group, which could be due to the higher ingestion of vitamin D supplements in the control group. These results are consistent with the results of *Marwashs et al*. study in which there was a direct correlation between vitamin D3 levels and MCV and age. They also showed that vitamin D and WBC count were linked to ethnicity [2]. Doudin et al. demonstrated a significant link between serum vitamin D3 levels and different RBC indices in German adolescent aged 11 to 17 years indicating an inhibitory influence of vitamin D3 on erythrocyte maturation [20]. Similar to *Marwah* and *Soliman* et al studies, we found no significant correlation between vitamin D3 level and RBC count, red cell indices such as Hb, HCT, MCH, MCHC, RDW, platelet count and PDW, and differential WBC count [2, 21]. As stated above, unequal gender distribution in the studied groups, sufficient sunlight exposure, and ingestion of vitamin D supplements may be probable causes. Although the percentage of female patients was higher than males in both subgroups of vitamin D3 deficiency (51.3% vs 30.9% and 12.2% vs 5.5% in subgroup with intermediate and low level of vitamin D3, respectively), but no significant difference was observed in vitamin D levels in both gender. Moreover, decreased levels of vitamin D3 were higher in female compared to males. It seems that despite the further decrease in vitamin D3 levels in females, the unequal

distribution of the gender in subgroups influenced the link between vitamin D3 levels and hematological indices and no meaningful difference was observed in hematological indices due to vitamin D3 deficiency. Furthermore, we did not observed a significant change in serum ALK levels between patients with abnormal levels of vitamin D3 and control as well as sup-groups of vitamins D3 deficiency. It was similar to a study conducted by *Shehla et al*. in which all the patients with vitamin D deficiency had normal levels of serum ALP activity. Also, there was no correlation between serum ALK levels and vitamin D3 levels in our studies. It was contrast to many studies in which a significant but reverse correlation reported between serum Vitamin D3 and ALK levels [19, 22, 23]. Baig et al. showed an increase in serum ALK levels of 19% of patients with vitamin D3 deficiency, as well (24). A likely reason for the lack of relationship between vitamin D3 deficiency and ALK levels found in our study is the unequal distribution of gender.

Conclusions

Generally, vitamin d3 deficiency may not have a significant impact on hematological parameters such as RBC count and indices (including MCH, MCHC, and RDW), Hb levels, HCT, differential WBC count, or platelet count and its indices (PDW). Suboptimal vitamin D3 also may not have an effect on the serum ALP concentration.

Limitation

Equal gender distribution of studied groups was not possible, but considering that the level of vitamin D3 in females was less than male, it seems that if there was gender equality in the groups, a significant difference may be observed in hematological or biochemical parameters. In addition, it was not possible to access information regarding to the consumption of vitamin D supplements by the participants.

Numb	**Abbreviation**	
1	**ALK**	Alkaline phosphatase
2	**Baso**	Basophil
3	**Eos**	Eosinophil
4	**DEA**	Di-ethanolamine
5	**Hct**	Hematocrit
6	**HB**	Hemoglobin
7	**Lym**	Lymphocyte
8	**MCH**	Mean cell hemoglobin
9	**MCHC**	Mean cell hemoglobin concentration
10	**MCV**	Mean cell volume
11	**Mono**	Monocyte
12	**Neu**	Neutrophil
13	**PDW**	Platelet distribution width
14	**RBC**	Red blood cell
15	**RDW**	Red cell distribution width
16	**WBC**	White blood cell

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