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Effect of Variation in Vitamin D Concentration on Some Immunological Markers in Single Males

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Abstract

Objective: This study was aimed to investigate the changes in some immunological markers with different concentrations of vitamin D. The parameters are immunoglobulin (IgA, IgM, and IgG,), anti-phospholipid (IgM, IgG), Anti-cardiolipin (IgM, IgG) and Erythrocyte Sedimentation Rate (ESR).

Methods: The specimens were collected from 90 volunteers. The samples were separated into three groups. The first group includes specimens with a normal value of vitamin D and the second group includes, people with marginal deficiency levels, as well as, the group with vitamin D deficiency. These tests were done on samples of ninety single males and their ages were 20-25 years. Serum Immunoglobulin (IgA, IgM, IgG) is detected by using Single Radial Immuno Diffusion (SRID). Enzyme-linked Immune Sorbent Assay (ELISA) technique was used to measure the concentration of vitamin D, anti-phospholipid (IgM, IgG) and anti-cardiolipin(IgM, IgG). Westergren method was used to measure Erythrocyte Sedimentation Rate (ESR).

Results: There was a change in levels of immunological indicators involved in this study. The differences were significantly decreased ($P<0.05$) in the case of IgA concentration but it was non-significant in the case of IgM, IgG. The present study showed that the APL (IgM, IgG) and ACL (IgM, IgG) slightly increased but it did not reach the level of significance. The level of ESR increased significantly ($P <0.05$).

Conclusions: Changes in immunological processes for many parameters that shared in the current study was associated with both marginal deficiency group and vitamin D deficiency group. Vitamin D deficiency also activates the inflammatory cells to increase and decrease of inflammatory indicators.

Keywords: Vitamin D; IgA; IgM; IgG; Anti-phospholipid; Anti-cardiolipin; ESR

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Introduction

Vitamin D deficiency is now recognized as a prevalent disease in the world. The major cause of vitamin D deficiency is less exposed to sunlight which is the main source of vitamin D for humans. Foods that are rich in Vitamin D are not provided or foods are fortified with Vitamin D is not enough to a child's or an adult's vitamin D requirement. The decrease in Vitamin D level deficiency causes the development of immune-related diseases such as autoimmune diseases [1,2].

Vitamin D is an essential element for many processes inside the body especially bone metabolism as it increases the intestinal absorption of calcium, which is important for skeletal health. Vitamin D is called a vitamin; on the other hand, it is a steroid pro-hormone acting in different places in the body. The vitamin D status of an individual is a result of both genetics and the environment. The major source of vitamin D is forming in the skin when exposed to the sun. In addition, vitamin D is absorbed from food and supplements. There are few foods have vitamin D. There are many factors that influence on the production of vitamin D in the skin; habits of sun exposure, types of clothing worn, age and latitude supplementation intake, skin pigmentation and different genotypes of vitamin D binding protein, as well as, enzymes may affect the vitamin D status [3-5]. Sim and colleagues have suggested that vitamin D has an important role in erythropoiesis and that deficiency brings a risk of anaemia with a lower concentration of haemoglobin and higher usage of erythrocyte-stimulating agents[6]. Vitamin D plays a major role in the modulation of the Immune system [7]. Immune components together with Vitamin D play a significant role in human health [2]. Vitamin D regulates the production of inflammatory cytokines and immune cells, which play a role in the pathogenesis of many immune-related diseases. This is because Vitamin D has receptors on immune cells (B cells, T cells, and antigen presenting cells, such as macrophages and dendritic cells) [7].

Vitamin D caused the decrease of inflammatory cytokines especially, IL-2 and interferon-gamma, modulating the differentiation and function of antigen-presenting cells (APC), thereby diminishing the activity of autoreactive T cells affects the stimulation of interleukin 4 production by T- helper 2 cells (Th2), and in the increasing of regulatory T cells, in particular, interleukin-10 (IL-10). It is important

to inhibit inflammation and decreasing the production of interleukin 17 (IL-17) by T- helper 17 cells (Th17). These mechanisms can play essential roles in reducing and ending inflammation [8,9]. Vitamin D has an effective role in preventing some types of diseases such as colon and prostate cancers, osteoporosis and diabetes type 1. Also, vitamin D has a relationship in chronic obesity-related diseases such as Mellitus diabetes, cardiovascular diseases and hypertension [10-12]. Anti-phospholipid syndrome (APS) is associated with the production of anti-cardiolipin (aCL) antibodies, which develop against the negatively charged phospholipids present in cell membranes. The syndrome may coexist with several, predominantly autoimmune diseases (secondary APS), or it may be present without any other disease. It has been reported that the incidence of aCL antibody positivity increases with age and concomitant chronic diseases [13-16]. A diabetic is an actual cause for atherogenic vascular diseases that arise from endothelial dysfunction, oxidative stress and inflammation. Immunological mechanisms might have a role in the pathogenesis of diabetic microangiopathy via immune complex deposition. Anti-cardiolipin (aCL) antibodies are a type of anti-phospholipid antibodies that binds to Cardiolipin in the presence of cofactor β2 glycoprotein 1(b2GPI). These (aCL) antibodies may promote ischemia and thrombosis through several mechanisms including functional alteration of protein C, impaired fibrinolysis, altered anti-thrombin level, inhibition of prostacyclin activity, platelet aggregability and complement activation [17,18]. Previous studies reflect the differences in the increased prevalence of antiphospholipid antibodies in types 1 and 2 diabetic patients [19].

Therefore, this research aimed to investigate some immunological markers including immunoglobulins, ESR, anti- phospholipids and anti-cardiolipin in different Vitamin D levels (enough, insufficient, and deficient) on single males. In this article, we address these issues with specific reference to the role of vitamin D in immune modulating.

Subjects and methods

Subjects

Ninety males at 20-30 years of age shared in this study. Women were excluded from this study. The samples were collected in Baghdad city during the period (February 2019). The groups were divided into three collections, the first one was samples with normal vitamin D concentration and the second one was people with normal marginal deficiency of vitamin D, as well as, group with deficiency concentration of vitamin D.

Methods

Under sterile conditions, the samples (7 ml from each participant) were collected in plain tubes. Sera were isolated by using centrifugation at 1000 g for 20 min at room temperature. Samples were immediately separated into aliquots and stored at (-20 C) until analyzed. The (SRID) method was used to estimate concentration of immunoglobulin (IgA, IgM, IgG). A sandwich type (ELISA) was used to measure the concentration of vitamin D, anti-phospholipid (IgM,IgG) and anti-cardiolipin (IgM ,IgG). Westergren method was used to measure ESR [20-28].

Vitamin D assessment: Serum levels of vitamin D were estimated by using ELISA test. In present study, vitamin D level that less than 20 ng/mL was considered as vitamin D deficiency. The levels of 20.1 to 29.9 ng/mL considered as marginal deficiency and higher than 30 mg/mL were considered as normal vitamin D levels [29-31].

This study was approved by scientific committee of Biology Department, College of Science, University of Raparin.

Statistical analysis

The data were analyzed using One-way Analysis of variance (ANOVA). The value of ($P<0.05$) was considered significant for all analysis's tests. A statistical process was performed by statistical Package for Social Science (SPSS) V22.

Results

Variation in immunological parameters shared in the current study; Ig((IgA, IgM, IgG,), APL (IgG, IgM), ACL (IgG, IgM) and (ESR) with different levels of vitamin D are given in the below tables (Tables 1-8) respectively. Significantly, IgA decreased ($P<0.05$) but the decreasing in both IgM and IgG were not significant as shown in table 1 and table 3 respectively. The slight increase in both of APL (IgG, IgM), ACL (IgG, IgM) was not significantly different as explained in table 4 to table 7 respectively. Changes in the level of ESR are given in table 8. There was significant increase ($P<0.05$) compared to the control group.

Discussion

An effect of variation in vitamin D concentration and immunological indicators was estimated via blood tests for three different groups of volunteers. The current study evaluated the impact of

Table 1: The means and standard error values of IgA concentration (mg/dl) at different concentration of Vitamin D.

		N	Mean ± Std. Error	F	P-value
IgA	Normal Vitamin D Group	30	92.8667 6.49470	23.189	0.002
	Marginal deficiency Vitamin D Group	30	76.9667 3.14660		
	Deficiency Vitamin D Group	30	46.1000 4.59021		

Where: The differences are significant at the 0.05 level.

Table 2: The means and standard error values of IgM concentration (mg/dl) at different concentration of Vitamin D.

		N	Mean ± Std. Error	F	P-value
IgM	Normal Vitamin D Group	30	88.4000 13.16219	0.102	0.904
	Marginal deficiency Vitamin D Group	30	84.0333 14.20344		
	Deficiency Vitamin D Group	30	79.8000 12.95737		

Where: The differences are significant at the 0.05 level.

Table 3: The means and standard error values of IgG concentration (mg/dl) at different concentration of Vitamin D.

		N	Mean ± Std. Error	F	P-value
IgG	Normal vitamin D Group	30	733.3333 171.30220	0.065	0.938
	Marginal deficiency vitamin D Group	30	766.6667 174.00511		
	Deficiency vitamin D Group	30	681.2667 160.57806		

Where: The differences are significant at the 0.05 level.

Table 4: The means and standard error values of ALP (IgM) concentration IU/ml at different concentration of vitamin D.

		N	Mean ± Std. Error	F	P-value
APL(IgM)	Normal Vitamin D Group	30	11.0000 0.57735	6.091	0.036
	Marginal deficiency Vitamin D Group	30	10.3333 0.88192		
	Deficiency vitamin D Group	30	13.3333 0.33333		

Where: The differences are significant at the 0.05 level.

Table 5: The means and standard error values of ALP (IgG) concentration IU/ml at different concentration of vitamin D.

		N	Mean ± Std. Error	F	P-value
APL(IgG)	Normal vitamin D Group	30	8.0000 0.57735	7.000	0.027
	Marginal deficiency vitamin D Group	30	10.0000 0.57735		
	Deficiency vitamin D Group	30	11.0000 0.57735		

Where: The differences are significant at the 0.05 level.

Table 6: The means and standard error values of ACL (IgM) concentration IU/ml at different concentration of vitamin D.

		N	Mean ± Std. Error	F	P-value
ACL(IgM)	Normal vitamin D Group	30	7.3333 1.20185	2.450	0.167
	Marginal deficiency vitamin D Group	30	8.3333 0.88192		
	Deficiency vitamin D Group	30	10.0000 0.10000		

Where: The differences are significant at the 0.05 level.

Table 7: The means and standard error values of ACL (IgG) concentration IU/ml at different concentration of vitamin D.

		N	Mean ± Std. Error	F	P-value
ACL(IgG)	Normal vitamin D Group	30	7.3333 0.33333	5.250	0.048
	Marginal deficiency vitamin D Group	30	8.3333 0.88192		
	Deficiency vitamin D Group	30	10.3333 0.66667		

Where: The differences are significant at the 0.05 level.

Table 8: The means and standard error values of erythrocyte sedimentation rate (mm/h) at different concentration of vitamin D.

		N	Mean ± Std. Error	F	P-value
ESR	Normal vitamin D Group	30	7.0000 1.73205	18.396	0.003
	Marginal deficiency vitamin D Group	30	12.0000 0.57735		
	Deficiency vitamin D Group	30	27.0000 3.78594		

Where: The differences are significant at the 0.05 level.

different vitamin D concentrations on some immune factors. Vitamin D has anti-inflammatory role [32,33]. A study shows that Vitamin D led to the decrease inflammation where Human hepatocellular carcinoma cell line, j774 cell line were induced inflammation by LPS (lipopolysaccharide) but given Vitamin D3 in different doses, caused the reduce of inflammation [7,34]. Human studies have explained that the higher levels of vitamin D had relationship with lower inflammatory markers including CRP, IL-6 and TNFα in healthy populations [35]. Vitamin D down regulates cellular response to tumor necrosis factor-alpha (TNF-α), B-cell antibody production, and pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-2, IL-6, IL-12, and interferon-gamma (IFN-γ) [36].

The present findings show that there was a decrease in the concentration of IgA in both marginal deficiency and deficiency groups compare to the normal vitamin D concentration group. The mean value for the normal vitamin D concentration group was 92.8667 mg/dl, whereas it was 84.0333 mg/dl, 46.1000 mg/dl for both marginal deficiency and deficiency groups respectively. Table 1 shows that the statistical analysis between values was significant ($P<0.05$).

For IgM level, the values dropped for both marginal deficiency group and deficiency group with 84.0333 mg/dl, 79.8000 mg/dl in comparison to the healthy group where it was 88.4000 mg/dl, as given in table 2 statistically, the differences were not significant. Table 3 shows that the means values of IgG concentrations were 733.3333 mg/dl, 766.6667 mg/dl and 681.2667 mg/dl for the normal vitamin D concentration group, marginal vitamin D concentrate on deficiency group and deficiency vitamin D concentration group respectively. Our study demonstrated that the concentration of both, APL (IgM, IgG) and ACL (IgM, IgG) increased but it was not significant. The mean values of APL for IgM were 11.0000 IU/ml, 10.3333 IU/ml, and 13.3333 IU/ml while mean values of APL (IgG) were 8.0000 IU/ml, 10.0000, 11.0000 IU/ml, for the normal vitamin D group, marginal vitamin D deficiency group and deficiency vitamin D group, respectively as shown in Tables (Table 4 and Table 5). The study reported that the results of healthy group, marginal group and deficiency group for ACL (IgM, IgG) were 7.3333 IU/ml, 8.3333 IU/ml, 10.0000 IU/ml for IgM and for IgG were7.3333 IU/ml, 8.3333 IU/ml, 10.3333 IU/ml respectively as given in tables (Tables 6 and 7). The results obtained from the current study revealed an increase in the mean values of ESR for both marginal vitamin D deficiency group and deficiency vitamin D group were 12.0000 mm/h, 27.0000 mm/h compared to normal vitamin D group where it was7.0000 mm/h (Table 8).

Conclusion

This work further supports that Vitamin D significantly reduced the inflammation. Therefore, this study strengthens the use of Vitamin D3 as proven effect on health-because of its normalizing action on inflammation markers.

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