Open Access Maced J Med Sci **electronic publication ahead of print,**
published on November 30, 2016 as http://dx.doi.org/10.3889/oamjms.2016.134

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
http://dx.doi.org/10.3889/oamjms.2016.134
eISSN: 1857-9655
Basic Science

The Hidden Function of Vitamin D

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Citation: Sibaii H, El-Zayat SR, El-Shaheed AA, Mahfouz NN, Sallam SF, El Azma MH. The Hidden Function of Vitamin D. Open Access Maced J Med Sci. http://dx.doi.org/10.3889/oamjms.2016.134

Keywords: Vitamin D; thymosin beta-4; immunity.

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Received: 10-Oct-2016; **Revised:** 10-Nov-2016; **Accepted:** 11-Nov-2016; **Online first:** 30-Nov-2016

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: There are no reports regarding the influence of vitamin D on thymosin ß4 and the cluster of differentiation CD4 levels which are important for maintaining a healthy immune system. Consequently, we aimed to explore this relationship through a study.

MATERIAL AND METHODS: The study was carried out on 35 subjects, screened for 25-hydroxy vitamin D[25 (OH) D] using ELISA method and they were divided into two groups: Group 1 consists of 10 healthy subjects with sufficient vit. D level > 24.8 ng/ml. Group 2 consists of 25 subjects suffering, severely, from vitamin D deficiency at level < 11.325 ng/ml. Also, Thymosin ß4, CD4 and zinc levels were performed.

RESULTS: There were significant differences between the two groups in the concentration levels of thymosin β4, as the group 1 has shown higher levels (P = 0.005). Whereas, CD4 and zinc levels didn't show any significant difference between the two groups. At the same time, a significant positive correlation has been observed between vitamin D, thymosin β4, and CD4 at (r = 0.719; P = 0.001), and (r = 0.559, P = 0.001) respectively.

CONCLUSION: We concluded that vitamin D may be an essential factor that influence or determine the level of thymosin β4. This study is the first that focused on demonstrating that sufficient level of vitamin D may have the ability to influence the thymic hormone thymosin β4 levels. Further studies on large scale of subjects are needed to explore the positive correlation we had found between vitamin D and thymosin β4 and CD4.

Introduction

The impact of Vitamin D has been observed in many aspects [1]. Regarding immune system and human health lower levels of vitamin D may cause of immune dysregulation [2]**.** In addition**,** Wei and Christakos in 2015 revealed that vitamin D may have immunomodulatory properties [3]**.** Vitamin D influences human health because vitamin D receptor and its activating enzyme 1-α-hydroxylase (CYP27B1) are expressed in activated lymphocytes, macrophages and dendritic cells [1]**.** This suggests an important impact of vitamin D especially in the field of human immunology [4-6]**.** Vitamin D intake is highly dependent on nutritional habits where 47% of vitamin D source come from food supplements [7]**.**

Thymosin ß-4 is one of the thymic hormones [8], it is abundant in human cells and tissues, representing 70–80% of the total thymosin content [9] [10] it is an active peptide with 43 amino acids [8] it is omnipresent as intracellular protein, bind to and sequester G-actin to modulate cell migration [11]**.** Several physiological properties of Tβ4 have been reported; [12] repairing and remodeling of skin, neural system and heart tissues following injury [13]**,** assisting in the development of B cells to plasma cells to produce antibodies [14] implicated in lymphocyte maturation and differentiation [9, 15]**,** controlling cell morphogenesis and motility [16] preventing fibrosis [17]**,** acting as a modulator of wound healing and inflammation [18] and regulating immunity [19]**.** Tβ4 is the major actin-sequestering molecule in all eukaryotic cells [20]**.** (Tβ4) is considered to play a significant role in the cellular metabolism due to its actin-sequestering properties [12]**.**

A cluster of differentiation cells- often referred to as CD4 cells- are glycoprotein located on the surface of various types of immune cells restricted to

T helper lymphocytes. It has an important function such as signal amplification and T- cell activation [21]**.** CD4 is a co-receptor that assists the T cell receptor (TCR) in communicating with an antigen-presenting cell [22].

Zinc was found to be necessary for a normal functioning of the immune system [23]**,** altered zinc levels disturb the functions of innate immunity [24]**,** and mild zinc deficiency depresses immunity [25]**.**

This is the first investigation to describe the relationship between vitamin D deficiency, thymosin ß4, and CD4 levels.

Subjects and Methods

Throughout the period from January to March 2015 subjects were recruited from the outpatient clinic of the Centre of medical excellence at the NRC, where 40 subjects were screened by full medical history, thorough clinical examination, nutritional questionnaire, and anthropometric measurement. Screened subjects were enrolled into the study according to the following inclusion /exclusion criteria:

Inclusion: Age ranging from 18-40 years, both sexes, suffering from easy fatigability and lethargy and marked Vit D deficiency < 12 ng/ml.

Exclusion: Systemic diseases (cardiac, hepatic, renal, pulmonary, etc) malignancy of whatever nature, any autoimmune disease.

Thus, 25 subjects (males, females) suffering from easy fatiguability and lethargy, of average age of 27.28 ± 1.5 were enrolled into the study as group), in addition to 10 age and sex matched healthy subjects having normal levels of Vit D > 30 ng/ml as a control group (group 2) .

Methods

Blood samples were drawn from all subjects to estimate complete blood picture. Plasma was separated for determination of vitamin D, thymosin β4, CD4 and trace element zinc .and stored at -80°C until used.

Plasma 25(OH)D, thymosin β4, CD4 levels were determined using a commercial enzyme immunoassay according to the manufacturer instructions (Glory Science Co., Ltd, 2400 veterans Blvd. Suite 16-101,Del Rio, TX78840,USA), performed at National Research Centre medical physiology department serum vitamin D sufficiency was defined as > 20 ng/ml and severely deficient < 12 ng/ml according to the committee to review dietary reference intakes for vitamin D and calcium [26]**.** Zinc levels were estimated by spectrophotometric method (Salucea, Haansberg 19, 4874 NJ EttenLeur, Netherlands).

Statistical analysis

Independent sample student's t-test (two tails)was used to determine the significant difference between the two groups and expressed as mean±SE Pearson correlations were used to analyse the association between 25(OH)D with CD4 and thymosin ß4, chi-square test for non-parametric data was performed to examine the relation between the number of subjects with energy and those lacking energy as a symptom of vitamin D deficiency.

Results

The total number of Group 1 was 10 (8 females and 2 males) and the total number of Group 2 was 25 (21 female and 4 males) as shown in Table 1.

Table 1: Gender distribution

Gender	Group 1 (n = 10)	Group 2 (n = 25)
Females	8 (80%)	21 (84%)
Males	2 (20%)	4 (16%)

Age and BMI are shown in Table 2.

Table 2: Age and BMI of subjects

Parameters	Mean ± SD Group 1 N = 10	Mean ± SD Group 2 N = 25	Significance p
Age years	28.5 ± 4.64	28 ± 7.51	P = 0.636 NS
BMI Kg/m^2	26.81 ± 3.89	27.41 ± 5.52	P = 0.717 NS

BMI:(body mass index); NS : Non-significant.

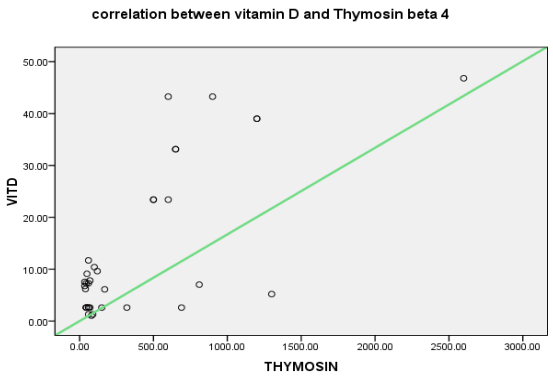
As shown in Table 3, group 1 had significantly higher 25 (OH) D levels in comparison to group 2 at ($p < 0.001$) the majority were deficient with 25 (OH) D levels. In addition there was a significant difference between thymosin beta 4 in group 1 where it has shown remarkable increase in comparison to group 2, in addition, an increased level of CD4 has been observed in group 1 in comparison to group 2 but this increase wasn't statistically significant, zinc levels didn't show any significant difference between the two groups.

Table 3: Comparison between Group I and II regarding vitamin D, CD4, Thymosin ß4, and Zinc

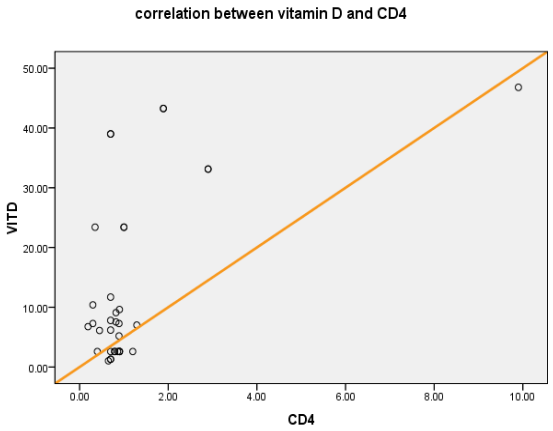
Parameters	Mean ± SE Group 1 N = 10	Mean ± SE Group 2 N = 25	Significance p
Vitamin D µg/l	34.77 ± 2.82	5.16 ± 0.63	P = 0.001
CD4 pg/ml	2.32 ± 0.88	0. 737 ± 0.05	P = 0.108NS
Thymosinß4 ng/ml	$9.400E_2$ ± 202	$1.844E_2$ ± 60.65	P = 0.005
Zinc µg/dl	$1.800E_2$ ± 9.35	$2.06E_2$ ± 17.63	P = 0.201 NS

p < 0.001 = very highly significant difference; CD4: Cluster of differentiation 4.

Figure 1 demonstrate the significant positive correlations of this short study where Figure 1A and Figure 1B represent the positive correlation between vitamin D, thymosin ß-4 and CD4 (r = 0.719, p = 0.001, r = 0.559, p = 0.001 respectively). Where Figure 1C has shown a significant positive correlation between thymosinß-4 and CD4 at r = 0.755, p = 0.001.



A)



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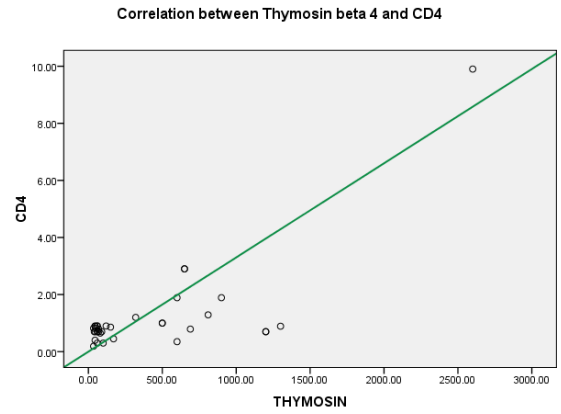

C)

Figure 1: A) positive correlation between vitamin D and thymosin ß-4 at r=0.719, p= 0.001; B) positive correlation between vitamin D and CD4 at r=0. 559, p= 0.001; C) A positive correlation between Thymosin beta 4 and CD4 at r=0.755, p= 0.001

The results of a chi-square test of independence: A chi-square test of independence was performed to examine the relation between the number of subjects with energy and those lacking energy (lethargy) as a symptom of vitamin D deficiency, is shown in Figure 2. Subjects with vitamin D deficiency suffer from lethargy than sufficient subjects, the difference was significant, χ^2 (1, N = 35) = 6.632, p < 0.001.

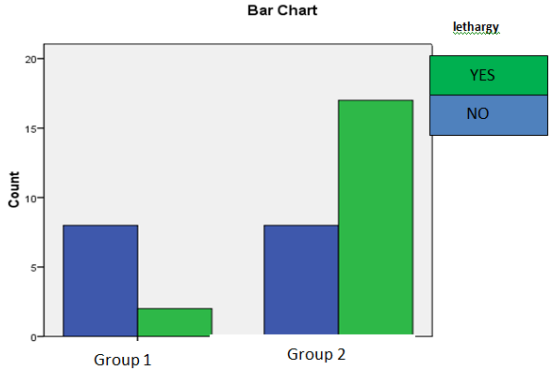

Figure 2: A bar chart showing the difference between Group 1 and 2 regarding lethargy

Discussion

In this study we identified that the majority of subjects were severely deficient in vitamin D where the ratio was 2:5 in which (28.58%) were sufficient and (71.42%) were severely deficient in vitamin D which could be attributed to blood sampling at the end of winter; an observation noted in the studies carried by both Anderson, and Pittawaw as they found vitamin D levels to depend on season [27] [28], because Vitamin D levels are in their lowest levels after winter and their higher at summer [29]. Our current study found a strongly significant correlation between vitamin D, Thymosin beta 4 and CD4. Thymosin beta 4 is the most abundant thymosin in human cells and tissues, it represents 70–80% of the total thymosin content [9] and implicated in lymphocyte maturation and differentiation [15] while vitamin D receptor VDR is found nearly in every tissue and cell type in the body [30] and resides in the cytoplasm in the absence of VDR ligands [31]. When stimulated with 1alpha,25-(OH)(2)D(2) or 1,25(OH)(2)D(3), VDR moves from the cytoplasm into the nucleus [31].

Thus, the strongly significant correlation between vitamin D, Thymosin beta 4 and CD4 found in this study may raise a speculation about a release of thymosin beta 4 secondary to vitamin D stimulated VDR in the thymus. The correlation found between vitamin D and CD4 could be explained that vitamin D has effects on adaptive immune cells because of the expression of the nuclear (vitamin D receptor) as well as vitamin D-activating enzymes in both T- and B-cells [32]. The VDR expression by these cells is very low in

resting conditions but when activated, T- and B cells up-regulate VDR expression significantly, allowing regulation of up to 500 vitamin D responsive genes which influence their differentiation and proliferation [33] [34] therefore leading to a shift from a proinflammatory to a more tolerogenic immune status [35].

A recent study by Hewison who proposed that vitamin D influence on T cells function by the direct conversion of 25(OH)D to calcitriol by T-cells, and the effects of calcitriol on T-cells in which calcitriol have indirect effects on antigen presentation to T cells [36]. This study also revealed a strong positive correlation between thymosin β4 and CD4in agreement with Knutsen and colleagues in 1999 [37] which could be attributed to the fact that thymosin β4 is the predominant form of thymic hormones [38], and that its primary function is to stimulate the production of T-cells which are targets of thymosin activity [39]. In our study vitamin D was in positive correlation with CD4 - that represent helper cells - which has found to contain the significant amount of VDR [40]. Our study was in agreement with (Ritterhouse et al) [41] vitamin D regulates T-helper 1 (Th1) and dendritic cell function [42], which suggest that vitamin D support the innate and the adaptive immune system.

We didn't find any significant difference regarding zinc levels between group 1 and 2 or any correlation between zinc and vitamin D, Thymosin beta-4 and CD4 because the sources of zinc like whole grains, cereals and legumes, was available for our subjects according to questionnaire; as whole grains are high in zinc [43]**.** In addition, a study conducted by Hess, 2007 revealed that zinc levels in the serum are not an indicator marker of zinc status because it is detectable in a population with risk and severe deficiency [44].

A recent study by Chiplokara and Kawade 2012 observed that zinc deficiency is very rare but moderate is widespread [45]. Vitamin D dietary intake is highly dependent on nutritional habits. However, a study with a global perspective found that 6 to 47% of vitamin D intake comes from dietary supplements [46] [7]. Thus, without supplementation, vitamin D status strongly will depend on endogenous vitamin D production which is also influenced by latitude, skin pigmentation, season, and lifestyle such as clothing [47] [48].

In conclusion, Vitamin D is obtained from limited dietary sources and the high vitamin D deficiency found in this study emphasises the importance of increased awareness and supplementation. It is apparent that vitamin D influences Tβ4 and CD4 levels so supplementation with vitamin D is essential to support immunity. More experimental trials in laboratories are needed to measure the levels of thymosin beta 4 in the compartments of thymus by its direct stimulation with vitamin D and measuring its concentration in vitro to explore the strong correlation found between vitamin D and thymosinβ4.

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