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ORIGINAL ARTICLE

Baseline levels determine magnitude of increment in 25 hydroxy vitamin D following vitamin D3 prescription in healthy subjects

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Abstract

Introduction Vitamin D deficiency is a major health problem which affects about one billion people in the world. Although, vitamin D supplementation is recommended as standard treatment of vitamin D deficiency, there are controversies on dose response relationship. In this regard, the present study aimed to determine the impact of vitamin D3 supplement on raising of serum 25 hydroxyvitamin D[25(OH)D] in healthy subjects with varying degrees of vitamin D deficiency.

Materials and methods In this clinical trial 114 subjects with varying degrees of vitamin D deficiency were entered and divided into three groups: serum levels of 25(OH) D less than 10 ng/ml, 10–20 ng/ml, and 20–30 ng/ml. All of the participants were given 50,000 units vitamin D3 per week for 8 weeks, thereafter, changes in serum levels of vitamin D and PTH were evaluated at week twelve. The results were analyzed using SPSS version 16 and $P < 0.05$ was considered to be significant.

Results Of the 114 vitamin D deficient subjects, serum level of vitamin D was below 10 ng/ml in 22 persons (19.3%), 10–20 ng/ml in 52 persons (45.6%) and 20–30 ng/ml in 40 persons (35.1%). Following vitamin D prescription all people with varying degrees of vitamin D deficiency obtained a favorable serum level. The increase in vitamin D levels were 26.4, 18.5, and 8.3 ng/ml, in individuals with baseline vitamin D levels below 10 ng/ml, 10–20 ng/ml and 20–30 ng/ml, respectively. The changes in 25(OH) vitamin D in all three groups were significant ($P < 0.05$), nonetheless no significant alterations in serum levels of PTH were observed ($P > 0.05$).

Conclusion Our results indicated an inverse relationship between baseline serum levels of 25(OH) D and its increment following treatment with vitamin D3. Therefore, the magnitude of increments in serum 25(OH) D is greater in subjects with lower baseline levels of 25(OH) D.

Keywords Vitamin D deficiency · Dose response relationship · Treatment

Introduction

Vitamin D deficiency is a major health problem. It is estimated that about one billion people in the world have vitamin D deficiency [1]. Vitamin D deficiency is also observed in apparently healthy people, as well as areas with high sunlight [2, 3]. Naturally, vitamin D3 (cholecalciferol) is produced by pro-vitamin available in the skin after sun exposure. Alternatively vitamin D2 (ergocalciferol) and D3 are absorbed into the body through gastrointestinal tract. Vitamin D3 (or D2) is converted to 25-hydroxyvitamin D (25(OH)D in the liver and then is transformed into active form, 1,25dihydroxyvitamin D(1, 25 (OH) 2D) by the enzyme 25 (OH) D-1 hydroxylase, in the kidney. As a hormone, vitamin D in association with fibroblast growth factor and parathyroid hormone (PTH) plays a major role in

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the calcium and phosphorus homeostasis [4]. Virtually, all cells express vitamin D receptor (VDR) and 25 (OH)-1α hydroxylase enzyme [5]; the latter is regulated primarily by the inflammatory signals and the growth stage of the cells that are unrelated to calcium concentrations in the extra-renal tissues [6]. Following to the binding of vitamin D to the VDRs, many metabolic pathways are activated, which ultimately promote the non-skeletal actions of vitamin D including the regulation of immune system and cellular growth and differentiation [7]. According to these findings, the association of vitamin D deficiency with increased prevalence of several diseases and higher mortality has been suggested [8–12]. Although there is much controversy regarding the role of vitamin D supplementation in the management of various disorders, most observations showed the effectiveness of vitamin D intake in the prevention of several chronic diseases [13–17] but not in others [18, 19]. Moreover, a recent study showed that the benefits of vitamin D supplementation are depends on a certain threshold of serum level of 25OHD [20].

Evidence has shown that the optimal minimum serum level of 25 (OH) D differs from 20 ng/ml for maintaining bone health to 40 ng/ml for prevention of cancers [21]. Nevertheless, there is actually no consensus regarding the optimal serum 25OHD levels for both bone health and extra-skeletal effects [22, 23]. Accordingly depending on the intended purpose, daily vitamin D doses from 600 to 10,000 IU have been recommended for treatment of vitamin D deficiency by various societies [24–27].

Although the results of previous studies proved to determine the magnitude of increments in serum level of vitamin D were inconsistent [28–34], it is likely that the increment of serum levels of vitamin D is higher in people with a lower serum level of vitamin D [31, 33, 35]. However, it is not well known if there is any difference in rising of serum vitamin D according to its initial serum levels.

The aim of present study was to determine increments in serum 25 OH vitamin D in otherwise healthy adults with various degrees of vitamin D deficiency following administration of a constant dose of Vitamin D.

Patients and methods

This clinical trial was performed on asymptomatic healthy subjects aged 20–40 years referring to Ali-ebn Abitaleb Hospital in Zahedan, Iran. The Zahedan City located in 29° 30′ N. The study was conducted during the fall and winter in 2017 and 2018.

All participants were selected from medical trainees, assistants, and workers with normal body mass index (BMI) that in term of sun exposure were in the same condition. After the initial evaluation, subjects with malnutrition, kidney, pancreatic or liver disease, use of medications affecting the metabolism of vitamin D3 (such as steroids and anticonvulsants) and supplementation of calcium and vitamin D3 in the last six months, hypercalcemia, sarcoidosis, any abdominal surgery, and kidney stones were excluded.

After an overnight fast 5 cc blood was obtained by venipuncture, and serum was separated by centrifugation. All of the samples were freezed in −80 degree centigrade until further analyses. 25-hydroxyvitamin D, and PTH were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun; Germany) and Intact PTH ELISA kit (Euroimmun; Germany), respectively. To exclude celiac disease as a potential cause of malabsorption, anti-TTG was measured by new generation TTG-IgA (AESKU.DIAGNOSTICS Company; Germany).

People with serum levels of 25 (OH) $D < 30$ ng/ml were enrolled in the study. In order to compare the level of vitamin D intake from diet, a nutritional assessment method as 24-h dietary recall (24 h) was used (two working and one weekend days). It was completed in the beginning and at end of the study and nutritional value was analyzed by N4 software program.

All participants were treated with oral vitamin D3 50,000 IU (Zahrawi Pharmaceutical Company) weekly for 8 consecutive weeks.

The serum levels of 25 (OH) D and PTH were again measured at the end of the 12th week. Adherence to treatment was monitored by phone call to participants weekly. The study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences and informed consent was obtained from eligible subjects (code: IR.ZAUMS.REC.1395.277).

Data analysis

To describe quantitative data, mean, and standard deviation, and to describe qualitative data, frequency, percentages, the charts were used. To compare the mean of the studied variables among the groups based on serum vitamin D levels, one-way analysis of variance (ANOVA) and Tukey post hoc test were used. If the data had non-normal distribution, using the Kolmogorov–Smirnov test, Kruskal-Wallis was used to compare groups. In addition, changes in serum levels of vitamin D before and after the intervention in each group were compared using the Wilcoxon sign rank test. Data were analyzed using SPSS version 16 (SPSS Inc., Chicago, IL, USA) and $P < 0.05$ was considered as the level of significance.

Results

A total of 204 participants were surveyed at the beginning of the study. After measuring serum vitamin D levels, 139 eligible individuals with vitamin D deficiency were included. The prevalence of vitamin D deficiency in the studied population was 68.13%, of which 12.44% had a vitamin D level <10 ng/ml.

Of 139 people, data of 114 subjects were finally analyzed and 25 subjects were excluded during the study due to pregnancy (3 persons), high anti-TTG levels (3 persons), poor adherence to treatment (8 persons), and loss of final assessment (11 persons).

Among the 114 participants, the mean age was 29.2 ± 6.2 years; 44 (38.6%) subjects were male and 70 (61.4%) were female. The number of males and females in groups <10 ng/ml, 10–20 ng/ml, and 20–30 ng/ml were 4 males (18.1%), 18 females (81.8%); 20 males (38.4%), 32 females (61.5%); and 20 males (50%), 20 females (50%), respectively.

The mean serum level of vitamin D was 17.6 ± 6.00 ng/ml (ranged from 16.3 to 29.8); 22 (19.3%) subjects had vitamin D levels lower than 10 ng/ml, in 52 persons (45.6%) vitamin D levels were between 10 and 20 ng/ml, and 40 individuals (35.1%) had vitamin D between 20 and 30 ng/ml. Baseline information of the participants is shown in Table 1.

There was a significant difference in dietary intake of vitamin D between two groups of < 10 ng/ml and 20–30 ng/ml ($P = 0.04$), however no significant difference was found between the other groups ($P > 0.05$).

Changes in serum levels of vitamin D and PTH are shown in Table 2. Changes in serum levels of 25 (OH) D per kilogram body weight in groups <10 ng/ml, 10–20 ng/mL, and 20–30 ng/mL were 0.44 ± 0.06, 0.3 ± 0.03, and 0.13 ± 0.02, respectively. The increments of serum vitamin D levels in each group are shown in Fig. 1. Serum levels of PTH did not differ significantly between study groups (Table 2).

Of 13 individuals whose serum level of 25OHD was higher than 50 ng/ml after treatment, 5(22.72%) were in group <10 ng/ml, 5(9.61%) in group 10–20 ng/ml and 3 (7.5%) in group 20–30 ng/ml, respectively.

Discussion

In relation to high prevalence of vitamin D deficiency it is crucial to delineate operative factors that mediate the response to vitamin D treatment. Some studies have suggested initial serum level of vitamin D as a potential contributing factor in the rising of serum vitamin D levels [31, 33, 35].

The present study was designed to investigate if there is any association between initial serum levels of vitamin D with its subsequent rising, following treatment. In subjects with varying degrees of vitamin D deficiency, before and after prescribing a fixed dose of vitamin D, the level of 25 OH D and its changes were measured and compared. The study groups were matched by diet, sun exposure, weight, and age. Individuals with serum levels of 25 (OH) $D <$ 10 ng/ml, 10–20 ng/ml, and 20–30 ng/ml showed the serum level increment of 26.4, 18.5, and 8.3 ng/ml, respectively.

In the current study, it was clearly shown that the increase in serum level of 25 (OH) D inversely correlated with its initial level of vitamin D which is in agreement of previous studies. Trang et al. showed an inverse relationship between the increase in 25(OH)D and the initial 25(OH)D concentration in both, cholecalciferol and cacifediol users groups. In cholecalciferol group after oral intake of 4000 IU vitamin D supplement for 14 days by individuals with baseline serum level of vitamin D, 4–13.6 ng/ml, 14–19.6 ng/ml, and 20–34.4 ng/ml, and increment in 25OHD was 12 ± 6.4 ng/ml, 10.4 ± 4.8 ng/ml, 5.2 ± 5.6 ng/ml, respectively [35]. In addition, results of a recent review showed an inverse albeit linear relationship between increase in serum vitamin D and baseline 25OHD serum levels after oral intake of cholecalciferol, however this relationship was not observed after oral intake of calcifediol [36].

Furthermore, Chel et al., in a study on the elderly people with a low initial level of vitamin D (8 ng/ml), reported an increase of upto 2.4 ng/ml for each microgram of vitamin D supplementation [29]. While Heaney et al. indicated increase serum levels of 0.4 ng/ml for each microgram of vitamin D in healthy young males whose initial mean levels were 28 ng/ml [31]. The great difference between the results

Table 1 Baseline data of the participant and daily dietary intake of vitamin D, calcium, and phosphor

Base line serum level of 25(OH) D				
Variable	<10 ng/ml Mean ± SD	10–20 ng/ml Mean ± SD	20–30 ng/ml Mean ± SD	*P* value
Age (year)	28.95 ± 4.4	28.98 ± 65	29.6 ± 6.5	0.86
BMI kg/m²	22.9 ± 3.4	24.7 ± 5.2	23.3 ± 4.1	0.19
Vitamin D (µg)	58 ± 28[a]	53.2 ± 28.9[a,b]	38.8 ± 23.8[b]	0.02
Calcium (mg)	802.6 ± 337.8	836.4 ± 200.9	813 ± 255.1	0.86
Phosphor (mg)	1022.7 ± 396.5	1041.5 ± 241.3	1080.8 ± 335.3	0.77

[a,b]Same letter in each row shows there is no difference between groups

Table 2 Serum level of 25 hydroxy vitamin D_3 (25(OH)D) and parathyroid hormone (PTH) before and after of vitamin D_3 consumption

Serum level of 25 (OH)D ng/ml	Serum level of 25(OH)D ng/ml					Serum level of PTH pg/ml				
	Before		After			Before			After	
	Mean Rank	Mean ± SE	Mean Rank	Mean ± SE	*p* value	Mean Rank	Mean ± SE	Mean Rank	Mean ± SE	*p* value
<10	11.5[a]	8.2 ± 0.52	59.20[a]	34.6 ± 3.53	< 0.001	72.77	76 ± 8.94	74.14	69 ± 6.02	0.34
10–20	48.50[b]	16.01 ± 0.35	59.16[a]	34.56 ± 1.99	<0.001	53.73	53.36 ± 3.68	55.29	54.46 ± 2.58	0.6
20–30	94.50[c]	23.29 ± 0.42	54.40[a]	31.65 ± 1.71	<0.001	54	54.32 ± 3.84	51.23	52.12 ± 3.01	0.12
p value	<0.001		0.76			0.054		0.77		

[a,b,c]Same letter in each column shows there is no difference between groups

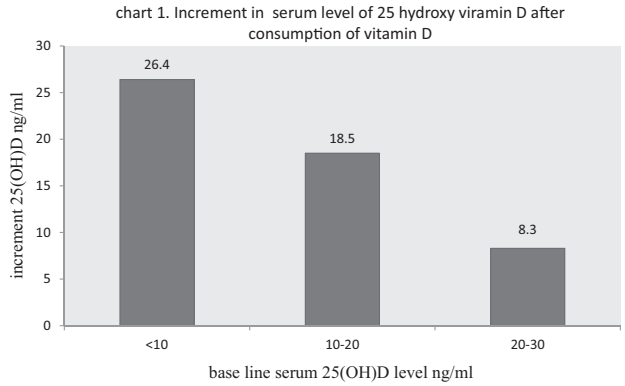

Fig. 1 Increment in serum level of 25 hydroxy vitamin D after consumption of vitamin D

of these two studies can be partly explained by initial serum level of vitamin D (8 ng/ml vs. 28 ng/ml).

While it is largely unknown how baseline serum levels of 25 OH Vitamin D determines its subsequent increment, a recently published study showed that increase in serum levels of free 25 OH vitamin D leads to proportional increase in 24, 25 (OH) 2 Vitamin D [37], which is in agreement with other studies [38]. According to these findings, higher baseline 25 OH Vitamin D results in higher free 25 (OH) Vitamin D which converts more 25 OH Vitamin D to inactive 24, 25 (OH) 2 Vitamin D.

Moreover, Genetic variations particularly in inactivating enzyme 24 hydroxylase have been postulated as an important determinant of response to vitamin D supplementation [39].

In a meta-analysis on 76 studies evaluating subjects over 50-years-old, 0.8 ng/ml/μg/day increase of serum vitamin D level was reported. In this meta-analysis, in concordance with our study, higher serum levels of vitamin D, as well as concomitant consumption of calcium were associated with lower increase in serum levels of 25 OH vitamin D [33].

Another factor influencing the response to vitamin D supplementation is digestive absorption. In the current study, healthy and young people without any malabsorption were selected in order to minimize the effect of this factor. In addition since celiac disease may present with atypical features [40], subjects with high serum levels of anti-TTG were excluded.

Finally fat mass was shown to affect changes in serum vitamin D level; e.g., individuals with BMI > 30 kg/m^2 require about two to three times more vitamin D supplementation [41]. In this regard Drincic et al. reported 2.5 U/kg vitamin D is required to increase 25 OH Vitamin D level in serum as much as 1 ng/ml [34]. In our study, subjects with normal BMI were enrolled, serum level changes of 25 (OH) D/kg body weight in groups <10 ng/ml, 10–20 ng/ml, and 20–30 ng/ml were 0.44, 0.30, and 0.13 ng/ml, respectively.

Thirteen out of 114 people achieved serum level of 25OHD above 50 ng/ml. However, the number of people with serum 25(OH)D level above 50 ng/ml, decreased from 5 subjects in groups of baseline serum level lower than 10 ng/ml and 10–20ng/ml to 3 subjects in group 20–30 ng/ml. Consequently, these results revealed the safety of an amplified supplementation in individuals with serum level of 25(OH)D 20–30 ng/ml, which confirms the results of previous studies [42, 43]. In addition no severe adverse events associated with higher serum 25OHD concentrations were confirmed by the Third National Health and Nutrition Examination Survey (NHANES III). Durazo et al. showed that all-cause mortality increased with 25OHD < 12.5 ng/ml, but this relationship did not correlate with the level between 12.5 ng/ml and 37.7 ng/ml [44]. These findings also signify that the assay of serum 25OH D probably is not necessary after treatment. In this regard, Cailet et al. disclosed frequent inappropriate 25OHD measurement in clinical practice [45].

A short study period can be considered as one of the limitations of the current study; therefore we suggest studies for more extended time, as well as on different age groups and among the persons with different BMIs. Another potential weak point of our study was more consumption of vitamin D by diet in the group with the serum level <10 ng/

ml in comparison to the group with 20–30 ng/ml. However, there was also a difference in the increasing vitamin D level among other groups with no difference in terms of dietary intake of vitamin D.

Conclusion

The results of the current study indicate that in subjects with different grades of hypovitaminosis D, response to a fixed-dose of vitamin D, is dependent to pretreatment level of 25 OH Vitamin D; the greater the vitamin D deficiency, the more increment in the serum vitamin D.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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