

Study to Evaluate Two Dosage Regimens of Vitamin D through an Academic Year in Middle School Girls: a Randomized Trial

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Abstract- Vitamin D is an essential hormone for growth and development of bones in children. There is a lot of evidence for deficiency of this vitamin in Middle East females. This study conduct to find a way to combat deficiency in girls during rapid growth phase of puberty in academic year. One hundred and two Middle School girls who had not consumed any vitamins supplement have been participated in this randomized clinical trial. They allocated randomly in two case groups who received 50,000 or 100,000 IU vitamin D3 in October and three months later in January or in control group who received vitamin E. At the end of winter blood samples for 25-hydroxyvitamin D were checked. The mean of 25-hydroxyvitamin D were 5.5 ± 1.5 ng/ml, 15.2 ± 6 ng/ml, 23.0 ± 6.8 ng/ml in control, 50,000 and 100,000 IU vitamin D groups respectively ($P<0.05$). Neither dosage of vitamin D could raise 25-hydroxyvitamin D above 20 ng/ml in all cases. However, none of the students in 100,000 IU of vitamin D3 had severe deficiency in winter. Headache, dizziness, and weakness were the most common complain after vitamin D consumption, but no difference between groups detected ($P>0.05$). Urine calcium/creatinin ratio was equal in case and control groups ($P>0.05$). 100,000 IU of vitamin D3 every three months (equal to 800IU/day) can raise 25-hydroxyvitamin D above 12 ng/ml in all cases but for area with high prevalence of sever deficiency, dosage more than 100,000 IU every three months or shorter interval recommended to achieve optimal level.

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Introduction

Vitamin D is an essential hormone for growth and development of bones in children and strong skeletal in adults (1). In vitamin D deficiency, only 10-15% of calcium of normal diet is absorbed this amount increases to the 40% in the presence of adequate vitamin D (2,3). Moreover, epidemiologic studies show vitamin D deficiency increases risk of important diseases like malignancy, cardiovascular disease, hypertension, stroke, diabetes, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease (4). Many studies in recent years have demonstrated that vitamin D deficiency can be existing in children and young adults without any obvious sign or symptoms (5-12). Girls particularly are more prone to vitamin D deficiency because of their less exposure to the sun and also limited

outdoor activities (5). Since about 35% of bone mass is acquired during 4 years around puberty (13) it seems rational to pay special attention to this group of age. Diagnosis, treatment and prevention of vitamin D deficiency in adolescents could, therefore, be very important for their future health (14). In Iran, primary evaluation demonstrated a high percentage of vitamin D deficiency in different groups of age and in various locations especially in winter (15-19).

In year 2007-2008, a study which was performed on girls aged 12-15 years living in Yazd demonstrated a 60% prevalence of vitamin D deficiency among this group of age (20). There are different ways to received sufficient vitamin D: either enough exposure to the sunshine or consume supplements separately or in combination with fortified foods. Considering the importance of puberty, in some countries (e.g. France

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and Argentina) in which the food are not fortified, vitamin D supplementations are offered to the girls in bolus doses every few months (18,21-23). In view of the high prevalence of vitamin D deficiency among girls in Yazd (20) this study was designed to determine the adequate amount of vitamin D is required for girls to maintain level more than 30ng/ml through academic year. Yazd province is one the sunniest province in the country and Middle East and therefore this study could reveal the minimum requirement of vitamin D in other area with less sunshine.

Materials and Methods

This study was conducted as a randomised clinical trial on the female students at Middle School (age between 12-15years) in Yazd, Iran, during autumn and winter of 2007. A total sample size of 102 subjects was calculated based on previous study (20), considering CI=95%, study power of 80%, SD1=4.4 ng/ml, SD2=4.6 ng/ml and minimal clinical differences ($d=3.3$ ng/ml) with lost of follow up 20%. The students were all healthy without any history of endocrine, bone, liver, kidney, gastrointestinal and metabolic diseases. None of the students had been consuming any vitamin D supplementations. Students were chosen randomly and after an explanatory session to describe the details of experiment to the parents and students, their informed consents were taken. Students were then arbitrary divided into three groups: 34 in control group, 34 in group I, and 34 in group II. A questionnaire was filled face to face for each of the student. Height, weight, and the stage of their puberty (tanner stage) were then assessed. In October 2007, one pearl of vitamin D3 (50,000 units, Alvahi Pharmaceutical Co, Iran) was given to group I and two pearls (100,000 units) to group II. The second and complementary dose (identical to dose one) was given to both groups three months later in January 2008. Control group in both phases received (vitamin E) as a control the number and shape of peals were completley identical to vitamin D pearls(one pearl in 50,000 group and 2 pearls in 100,000 group). An information pack on vitamin D side effects with a questionnaire was provided for participated to fill if any

of side effects occurred within two weeks of taken doses. After two weeks all the questionnaires were collected and also a sample of urine was taken to be assessed for hypercalciuria. The urine samples were kept in acid wash containers and immediately were transferred to the laboratory to be examined for calcium and creatinine using colorimetric method. One month after taken second dose, 3-5 ml blood sample was taken and assessed for 25-hydroxyvitamin D (25-OH Vit D) . The blood samples were transferred to the laboratory where they were centrifuged and freeze at -20°C . Chemiluminescence immunoassay (DiaSorin, LIAISON® 25-OH Vitamin D assay) was used to measure 25-hydroxyvitamin D (as a main indicator of vitamin D). SPSS software was used to analyze data where statistical descriptive & analytic tests like mean \pm SD, ANOVA and Tukey's test were applied. $P<0.05$ was considered as the significant level of differences.

Results

This study was performed on 102 female students with mean age of 12 ± 0.84 . During the course of study 5 students were dropped from the study because of either their migration to the other city or their absence at the time of second dose or sampling. One student also died because of car accident during the course of this study. Ninety six remaining students were as following: 31 in control group, 34 in group I, and 31 in group II.

The mean and SD of 25-hydroxyvitamin D was 5.5 ± 1.5 ng/ml in control group(range4-7), 15.2 ± 6 ng/ml (range7-34) in group I which received 50,000 units vitamin D3, and 23 ± 6.8 ng/ml (range13-37) in group II with 100,000 units vitamin D3. The differences between control group and group I as well as group I and II were reached significant level ($P<0.0001$).

Table 1 shows distribution of patient according to severity of vitamin deficiency after vitamin D supplement.

Neither of vitamin D dosages could raise the serum level of 25-hydroxyvitamin D above 20 ng/ml in all cases. However, none of the students in group 2 who received 100,000 units of vitamin D3 had sever deficiency (less than 10ng/ml).

Table 1. The distribution of the severity of vitamin D deficiency among treated groups.

	Vitamin E (control) (I)	Vitamin D 50000 unit (II)	Vitamin D 100000 unit (III)
Sever deficiency (25(OH) D<10)	%100	13.5%	%0
Deficiency 25(OH) D=10-20	-	%73	%45
Adequate 25(OH) D=20-30	-	10.8%	35.5%
Ideal 25(OH) D=30-100	-	2.7%	19.5%
$P<.0001$	$P<0001$ group I&II	$P<0.01$ group II&III	$P<0.001$ group I&III

Table 2. Relative distribution of hypercalciuria in different treated groups.

Mg Ca/mg Cr	Range	Mean & SD
Placebo	0.02-0.17	0.03 ±0.07
50000 IU	0.02-0.23	0.03 ±0.08
100000 IU	0.02-0.2	0.04 ±0.08

P>0.5

Two of participants; one in control group and one in group 1, demonstrated hypercalciuria with normal serum level of 25-hydroxyvitamin D and calcium blood level (*P*>0.05). This result is shown in Table 2.

The most common side effects were reported by the participants were: headache, dizziness, and weakness D following consumption of vitamin D supplements. However, the differences between these side effects and the various dosage of vitamin D and placebo did not reach significance (*P*>0.05). Also no significant differences were observed between BMI and tanner stage with 25-hydroxyvitamin D levels.

Discussion

It has been suggested that a serum level more than 11 ng/ml provides enough vitamin D to prevent acute manifestation of deficiency on bone and increasing this amount up to 90 ng/ml would not produce any toxicity. Although the ideal blood level of 25-OH Vit D are still unknown, but higher level of 25-OH Vit D (more than 30 ng/ml) is required to control secondary hyperparathyroidism and to increase calcium absorption (14). Because of vitamin D unique pharmacokinetics model, serum level higher than 40ng/ml is essential to saturate 25-hydroxylase enzyme and to optimize the kinetics of 25-OH Vit D production⁽¹⁵⁾. Although the exact optimal consumption dose of daily vitamin D is not lucid, 2000 units has been suggested to be safe for children (21). Our study, however, demonstrated that in population with more than 50% prevalence of vitamin D deficiency like many middle east country 50,000 units of vitamin D every three months, which is equal to 400 units daily, only in 13% of cases is sufficient and in only 3% is ideal. Even 800 units of vitamin D daily (100000IU/three months), which is double the amount has been suggested for sunny areas, manage to produce ideal serum level of vitamin D only in 20% of cases (at the end of the winter) and in 45% of cases prove to be insufficient. In a study was carried out on French adolescences aged 10-15 years, three doses of 100,000 units of vitamin D every three months from the beginning of the school were able to raise their serum

level of vitamin D above 20 ng/ml with decreasing their parathyroid hormone levels. This study is very similar to our study regarding the duration and the age of students participated. However, despite the presence of some students with sever deficiency in their samples; they managed to increase the serum level of vitamin D above 20 ng/ml in all cases. This discrepancy could partly be due to either the consumption of one extra dose of vitamin D in their study or utilization of additional fortified foods or magnitudes of sever vitamin deficiency in our study (23).

In another study was conducted in Argentina(latitude -55 degree), two doses of 100,000 units of vitamin D was supplied every three months to the children aged 7±4Y it was able to produce enough serum level of vitamin D at winter. However, in that study the age of participated was lower and the average primary serum level of vitamin D was higher (29±6 ng/ml) (22).

Our study which was performed on older girls who had been receiving no vitamin supplementations with more sever primary deficiency might explain this inconsistency. In another study in Argentina, even 150,000 units of vitamin D was claimed to be sufficient in autumn and winter (24). In conclusion, above study demonstrated that 100,000IU/ three months of vitamin D is able to prevent acute bone problems at this age (25 OH Vitamin D>12 ng/ml), but in growing girl population with more than 50% vitamin D deficiency, like many middle east country usual recommendation of vitamin D is insufficient and it is necessary to implement additional means; such as reducing the interval of bolus doses to 2 months instead of every three months or applying an initial loading dose to conquer the sever deficiency and then having a maintenance dose every three months, to provide enough level of vitamin D.

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