

## Effect of different doses of parenteral vitamin D3 on serum 25 (OH) D concentrations

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### ABSTRACT

*Background and the purpose of the study:* Parenteral Vitamin D3 is commonly prescribed in some developing countries like Iran and data about its effects on serum 25(OH)D concentration are scanty. Current study was designed to evaluate the effects of different doses of parenteral vitamin D3 on serum 25(OH)D concentration.

*Methods:* Forty two healthy volunteer were selected and randomly assigned into 3 groups. Groups I and II received 300000 and 600000 units of intramuscular vitamin D3 and group III received placebo. Serum 25 (OH)D concentration were evaluated before, 2 weeks, 2 months and 4 months after injection.

*Results and major conclusion:* Serum 25(OH)D in groups I and II were significantly higher than those before injection ( $<0.001$ ). At the end of the study, serum 25(OH)D concentration in groups I, II, and III were  $48.20 \pm 28.32$  ng/ml,  $65.46 \pm 33.52$  ng/ml, and  $14.38 \pm 11.14$  ng/ml, respectively. Relative frequency of serum 25 (OH) D above 80 ng/l in groups I, II and was %20 and %33.3 respectively. One case in group I and one case in group II (in two sessions) showed 25(OH)D concentration above 100 ng/ml. Vitamin D injection especially at doses higher than 300000 IU may be associated with 25(OH)D concentration higher than the accepted normal values.

**Keywords:** Vitamin D3, 25(OH) D, Hypervitaminosis D

### INTRODUCTION

In recent years numerous studies have revealed high prevalence of vitamin D deficiency in developing countries and its prevalence in Iran is estimated about %44 - %79 in different studies (1,2).

Assay of the serum 25(OH)D in Iran is limited to research in a few clinical laboratories in major cities and is not available as a routine diagnostic test. Therefore in most cases, parenteral vitamin D3 is prescribed solely based on clinical suspiciousness and/or serum concentration of calcium, phosphate and alkaline phosphates. Vitamin D deficiency can causes wide spectrum of clinical presentations from musculoskeletal pain to severe bone deformity (3-5), which are neither sensitive nor specific for diagnosis of vitamin D deficiency. As a result, blind administration of high doses of parenteral vitamin D in short interval which is a common practice in Iran is blindly and there are limited data on its therapeutic and toxic effects.

Previous studies have shown delayed and long duration response of serum 25(OH)D concentration by intramuscular injection of vitamin D3 (6-8) and it has been reported that response to oral vitamin D

products is more predictable (9).

Most studies have not compared different doses of parenteral vitamin D and the Present study was designed to evaluate the magnitude and duration of different parenteral doses of vitamin D3 on serum 25(OH)D concentration.

### MATERIALS AND METHODS

#### *Study's population and classification*

This study was conducted during winter of 2006 as a double-blind clinical trial in Qazvin city located in north-east of Iran at latitude 50 degree. Healthy subjects of 20-60 years old from healthy staff working in Boali hospital (internal medicine center of Qazvin) and their relatives were enrolled in the study. Volunteers were fully informed about the study protocol before the study and written consent was obtained. The study protocol was approved by the Ethics Committee of University of Medical Science of Qazvin. Participants using drugs containing vitamin D products, calcium, anticonvulsants, estrogen and diuretics and those who had used parenteral vitamin D products during last 6 months were excluded. Basal serum 25(OH) D concentration was

measured and those with serum 25(OH) D above 75 ng/ml were excluded (10).

Volunteers were randomly assigned to 3 groups and were subjected to injection of vitamin D3 in the first half of December 2006. Group I participants received 300,000 IU vitamin D3 in one side gluteus muscle and placebo in other side, group II participants received 600,000 IU vitamin D3 (300,000 IU in each gluteus muscle) and group 3 received placebo in each gluteus. Volunteers and laboratory staff were blind about assignment.

#### Laboratory measurement

Five ml of blood was drawn in no fasting status before injections, 2 weeks, 2 months and 4 months after injections and samples were taken immediately to the laboratory, centrifuged within 30 minute of collection and saved below - 20 °C. After completing the study all serum samples were analyzed for 25 (OH) D concentrations. Serum 25 (OH) D concentrations was measured by RIA (radioimmunassay) method using DRG Kits. Normal range of 25(OH) D was 10- 75 ng/ml. Intraassay and interassay coefficient of variation were reported %7.9 and %8.2, respectively.

#### Statistical analyses

Serum 25(OH) D concentration before injection, 2 weeks, 2 months, and 4 months after injection was represented as mean  $\pm$  SD in each group and session. Serum 25(OH) D concentration of post injection samples were compared with pre-injection ones using repeated analysis of variance (ANOVA). Significance was considered at the level of  $p < 0.05$ .

Relative frequency of hypervitaminosis D was calculated in groups I- III in each session separately using 2 cutoff for definition of hypervitaminosis D: 25(OH) D > 80 ng/ml and 25(OH) D >100 ng/ml. SPSS 13 statistical software package was used for analysis.

### RESULTS

Results are from 33 participants. Nine volunteers dropped out from the study which for each group is shown in table 1 separately. There were no significant differences in baseline characteristics of participants including age, gender, and BMI (Table 1). Baseline serum 25 (OH) D concentration of participants dropped from the study showed no significant differences with those who completed the study (Table 1). Overall %61 of participants had vitamin D deficiency (25(OH)D  $\leq$ 20 ng/ml) (10). Mean serum 25(OH) D concentration 2 weeks after vitamin D3 injection didn't show any significant difference with the pre-injection serum 25(OH) D concentration in each group.

Mean serum 25(OH) D concentration 2 and 4months after injection increased significantly in groups I and II compared with pre-injection concentration. At the end of the study (4 months after injection) mean

25(OH) D in groups I II and III were  $48.20 \pm 28.32$  ng/ml,  $65.46 \pm 33.52$  ng/ml and  $14.38 \pm 11.14$  ng/ml respectively (Table 2) .

At the end of the study serum 25 (OH) D continued rising in groups I and II (Figure 1). Groups I and II showed a mean increase of 23.8 and 45.15 ng/ml while the placebo group showed a mean decrease of 1.38 ng/ml compared with baseline concentration.

Distribution of serum 25(OH) D concentrations is shown in figure 2. Cases with 25(OH) D above 80 ng/ml (200 nmol/l ) was detected as early as 2 weeks after injection and also in later phases. Highest relative frequency of high 25(OH) D concentration in group I was about %20 ( at 2nd month of the study)and in group II was %33.3 (at the 4th month of the study). At the end of the study one case in group I and 1 case in group II (in two sessions) developed 25(OH) above 100 ng/ml (250 nmol/l). pre-injection serum 25(OH) D concentration in cases which developed 25(OH) D above 80ng/ml had no significant difference with cases with lower serum 25(OH) D. Indeed, %30 of cases who developed high 25(OH) D had vitamin D deficiency before injection.

### DISCUSSION

Results of the present study indicates late and long lasting effects of intramuscular vitamin D3. Two weeks after IM injection serum 25(OH) D concentration was not significantly different from pre-injection concentration.

Few studies have been designed to evaluate the effects of parenteral vitamin D on serum 25(OH)D concentration. Whyte et al studied the effects of 80  $\mu$ g/kg and 200  $\mu$ g/kg doses of parenteral vitamin D on serum 25 (OH) D on 3 and 6 healthy people respectively. One week after administration serum 25(OH) D began to rise which continued until the end of the study (7 weeks). On the other hand, serum 25(OH) D began to increase during 4- 8 hrs after oral administration and maximum serum concentration was achieved 1 week after drug administration(6).

Regarding the late onset effect of parenteral vitamin D, this product doesn't seem suitable for patients with symptomatic vitamin D deficiency. Regarding to high vitamin D storage in fat tissue, it is expected that intramuscular administration of high dosage of this vitamin induce long term effects on serum 25(OH) D concentration. In the present study the curve of serum 25(OH) D concentration was still rising 4 months after injection in groups I and II. In another study, 600.000 IU Vitamin D administered and serum 25(OH) D was evaluated after 4 and 12 months. Serum which were significantly higher after 4 ( $114 \pm 35$  nmol/L), and 12 months ( $73 \pm 13$  nmol/L) compared with baseline ( $32 \pm 8$  nmol/L) ( $P < 0.001$ ) and increased by an average of 128% over the 12 months (7).

**Table 1.** Basal characteristics of volunteers in 3 groups of the study

Variables	Group I	Group II	Group III
Male, number	3	5	2
female, number	11	9	12
Total, number	14	14	14
Drop out, number	2	1	6
BMI (Kg/m <sup>2</sup> ), mean (SD)	25.0(3.6)	23.8(4.1)	24.6(4.8)
Age (years), mean (SD)	38.7(10.2)	33.8(9.4)	33.0(11.2)
Serum 25 (OH) D at beginning the study (ng/ml), mean(SD)	27.24(21.30)	25.20(17.09)	25.10(14.4)
Serum 25(OH)D completed the study (ng/ml), mean(SD)	24.40(18.39)	20.31(13.07)	15.72(10.43)

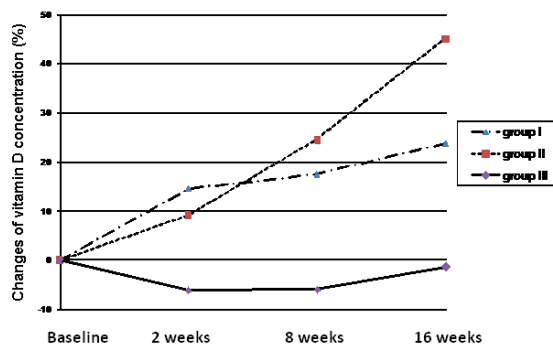
BMI-body mass index

Group I :300.000 unit vitamin D3, Group II :600.000 unit vitamin D3, Group III: placebo

**Table 2.** Changes of serum 25(OH)D in each session

Groups	Baseline	2 weeks	8 weeks	16 weeks	p
Group I, mean (SD) (ng/ml)	24.40±18.39	39.01(28.75)	46.25(21.16)	48.20(28.32)	P<0.001*
Group II, mean(SD) (ng/ml)	20.31±13.07	27.33±27.86	45.42±28.14	65.46±33.52	P<0.001*
Group III, mean(SD) (ng/ml)	15.72±10.43	10.14±2.54	9.75±5.94	14.38±11.14	NS

Serum 25 (OH) D concentrations at 8 and 16 weeks after injection were significantly higher than baseline concentration in groups I and II. Concentration at 2 weeks did not show any significant difference with baseline level in any group.

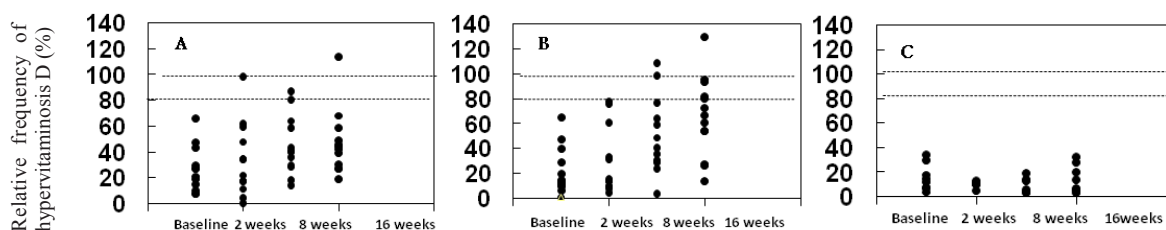
**Figure 1.** Changes of vitamin D concentration in different time that were assessed after injection.

In Heikinheimo study, an annual intramuscular injection of ergocalciferol (150,000 IU) normalized low serum 25(OH) D concentration in elderly people for 1 year (8). Stephens and colleagues treated a group of vitamin- D- depleted Asians with oral or intramuscular 100000 IU ergocalciferol. Treatment produced a sustained rise in the serum concentration of 25- hydroxyvitamin D, which lasted for 6 months. The response was more predictable after oral compared with intramuscular

administration (9).

In this study mean serum 25(OH) D increased at maximal drug effect (4th month) at 300,000 IU, and 600,000 IU doses about 23.80±19.9 ng/ml (CI 9.54-38.05) and 45.15±27.10 ng/ml (CI 28.77-61.53), compared with pre-injection concentration respectively. On the other hand, mean serum 25(OH) D of placebo group was less than baseline (-1.38±6.96 ng/ml) at 4th month of the study. Mean increase of serum 25 (OH) D in group who received 600000 IU was about 2 times greater than those of 300000 IU group.

Definition of hypervitaminosis D is somehow vague and different cutoff points of serum 25 (OH) D has been set for its definition, however all instances of vitamin D toxicity have been associated with serum 25(OH) D concentration higher than 80 ng/ml or 200 nmol/l (11). Therefore most of the researchers set the cutoff point of hypervitaminosis D serum level 25(OH) D at least 200 nmol/l (11,12). Using the cutoff point of 200 nmol/l, relative frequency of hypervitaminosis D in this study was about 20 and 33%, in group I and II respectively. The most notable point is occurrence of the highest frequency of hypervitaminosis D at 4th month of study in group II. This study ended at 4 month, so it is entirely



**Figure 2.** Relative frequency of hypervitaminosis D in groups I to III in different sessions of the study. Dashed lines represent two different cut offs for definition of hypervitaminosis D. A: group I , B: group II , C: group III

possible that high frequency of 25(OH) D concentration > 200 nmol/l continue in latter months.

### CONCLUSION

According to the results of this study parenteral administration of 300000 or 600000 units of vitamin D3 can be associated with 25(OH) D concentration that exceed the upper limits of normal and may causes hypervitaminosis D.

In Iran, vitamin D is commonly prescribed as intramuscular injection and in most cases it is prescribed without 25(OH) D concentration assessment and before vitamin D deficiency confirmation. Regarding high frequency of serum 25(OH) D above 200 nmol/l after parenteral administration, prescription of this product could be harmful even in vitamin D deficient subjects (in this

study %30 of cases who developed hypervitaminosis D were vitamin D deficient before injection).

However in most countries parenteral vitamin D are not used and maximum drug dosage is 50,000 IU per each oral product which is used for treatment of vitamin D deficiency.

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