Treatment of Hypovitaminosis D With Pharmacologic Doses of Cholecalciferol, Oral *vs* Intramuscular

An Open Labeled RCT

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Abstract and Introduction

Abstract

Objective Vitamin D deficiency is a worldwide health problem. Usual supplements are inadequate for prevention of hypovitaminosis D, and much higher doses are needed for its treatment. This study was designed to compare the efficacy and practicality of high-dose intramuscular and oral cholecalciferol in treatment of hypovitaminosis D and to evaluate durability of the effect of each remedy.

Design Ninety-two patients with hypovitaminosis D [serum 25(OH) D level < 75 nmol/l] were enrolled in a randomised clinical trial. Participants were randomly assigned to receive 300 000 IU cholecalciferol, either intramuscularly as a single injection or orally in six divided doses during 3 months period. Serum 25(OH) D level was measured at baseline and at 3 and 6 months.

Results Both treatment regimens significantly increased the serum 25(OH)D level. Delta change in serum 25(OH) D level from baseline (presented as mean ± SEM) at month 3 was significantly higher in oral than injection group (90 ± 11·2 and 58·8 ± 8·9 nmol/l, respectively, $P = 0{\cdot}03$); but was similar at 6th month intervention (52·1 ± 7·6 and 62·2 ± 6·7 nmol/l, respectively, $P = 0{\cdot}32$). There was a marginally significant trend in favour of oral group in the proportion of cases attained vitamin D adequacy at 6th month ($P = 0{\cdot}06$); but still 15% of all patients remained at < 50 nmol/l.

Conclusion Both regimens were considerably effective, safe and practical in treating hypovitaminosis D. Although we revealed superiority of oral route, at least at early short time, the way of treatment may depend on the patient's choice, compliance and availability of various forms of the drug in any regions.

Introduction

Vitamin D deficiency is now recognized as a pandemic.[1–8] It causes rickets in children and may precipitate or exacerbate musculoskeletal pain, fibromyalgia, osteopenia, osteoporosis and fractures in adults. Vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, infectious diseases and even depression.[9–15] The major cause of vitamin D deficiency is inadequate exposure to sun light. Wearing sunscreen significantly reduces vitamin D synthesis in the skin. Very few foods naturally contain vitamin D and vitamin D-rich foods are often inadequate to satisfy vitamin D requirements. Using serum 25(OH)D level, measured by a reliable assay, is the standard test for vitamin D status. Although some controversies exist regarding the optimal level of serum 25(OH)D, based on its associations with PTH levels, and studies of calcium absorption and bone density, serum 25(OH)D may be classified as three different categories: deficiency, a 25(OH)D of < 50 nmol/l; insufficiency, a 25(OH)D of 50–74 nmol/l; and sufficiency, a 25(OH)D of 75–250 nmol/l.[16–22] Improving vitamin D status may have some positive effects on public health and may reduce health care costs for many chronic diseases.[23]

Despite its widespread deficiency and the increasing awareness of vitamin D importance in general health, there is little evidence available for its treatment. Cholecalciferol (D_3) and ergocalciferol (D_2) are equally effective in raising 25(OH)D level.[24] It has been shown the usual supplements are inadequate even for prevention; and much higher doses are needed for treatment. A physician may choose between high-dose oral or, if available, injectable forms of vitamin D; although the later form is unavailable in some developed countries. There are many unanswered clinical questions in using different pharmacoceutical forms of vitamin D, and because lack of comparing studies, it is even unclear whether to treat orally or by injection.

Considering all of these uncertainties, we conducted this head to head comparison study to evaluate oral and injectable routes in treatment of hypovitaminosis D. We aimed to assess the efficacy of each method, using the same dose of 300 000 IU D_3, in achieving normal serum 25(OH)D level, the durability of the response, the practicality and the possible toxicity.

Materials and Methods

Participants

This randomized clinical trial was conducted on outpatients with diagnosis of osteoporosis, osteopenia, fibromyalgia and nonspecific musculoskeletal pain visited in the Rheumatology clinic of Firouzgar Hospital, Tehran, Iran, between October 2010 and March 2011. Eligible participants were those with serum 25(OH)D level of <75 nmol/l aged 20–70 years. Patients were excluded if they had hypercalcaemia, primary hyperparathyroidism, Paget disease, thyrotoxicosis, pregnancy, active malignancy, hypercalciuria, history of liver disease, renal insufficiency, clinically apparent malabsorption syndrome, using drugs known to affect vitamin D metabolism (anticonvulsants, glucocorticoids) or receiving any form of supplements containing vitamin D during the study. Volunteers were fully informed about the study protocol, and written consent was obtained. The study was approved by the Tehran University of Medical Sciences Ethnic committee and was registered with Iranian Registry of Clinical Trials (IRCT201010174950N1).

Protocol

Of 150 patients assessed primarily for eligibility, 92 patients were randomly assigned to either intramuscular injection (group 1) or oral (group 2) treatment by using 4-block randomization method. Group 1 received a single intramuscular D_3 injection (1-ml ampoule, 300 000 IU/ml in sesame oil, made by Caspian Pharmaceutical, Iran) at the study entry. The second group received the same total dose of 300 000 IU D_3 in the form of six pearls, each containing 50 000 IU D_3 (D-Vitin, Zahravi Pharmaceuticals, Iran) as follows: the first pearl was delivered at study entry, followed by one pearl weekly for another 3 weeks and then monthly for 2 months. Baseline doses were administered by study personnel on site for both groups, resulting in 100% compliance. Compliance with subsequent doses in the second group was assessed by pearl count and accepted if ≥ 80%.

The patients were asked to come back again at 3 and 6 months after the first dose of drug. To achieve the best results, all of participants were called by phone 1 week prior to their next appointment. Age and BMI were recorded; and a check list including weekly food frequency, daily sun exposure and possible extra-protocol vitamin D usage was filled up for the patients at each visit.

Main outcome was delta change in mean serum 25(OH)D level and also achievement of serum 25(OH)D ≥ 75 nmol/l at three and 6 months (±1 week) after the study entry. In addition, frequency of serum 25(OH)D ≥ 50 nmol/l and also > 250 nmol/l were assessed. In case of occurrence of 25(OH)D level > 250 nmol/l at any time during the study, serum calcium and 24 hours urinary calcium excretion were measured. In all cases remained vitamin D deficient at 3rd month intervention, we performed a serum anti-tTg assessment to rule out asymptomatic malabsorption.

Laboratory Assessment

Serum samples were stored at −20°C. Fasting serum 25(OH)D level was measured by trained technicians of a single laboratory at baseline, and at 3 and 6 months after the first dose of vitamin D_3. Serum 25(OH)D was measured by radioimmunoassay (Immune diagnostic systems, Boldon, UK) according to international instructions. Specificity and sensitivity for 25(OH)D measurement were 100% and 5 nmol/l, respectively. Inter-assay and intra-assay coefficients of variation were 6·4 and 5·6% at 175 nmol/l, respectively.

Statistical Analysis

Data analyses were performed according to a per-protocol analysis using 'last observation carried forward' (LOCF) method, in which missing data at month 6 were replaced by imputation of values of month 3. Numeric variables are presented using mean and standard error of mean (SEM). Independent sample *t*-test was used to compare baseline characteristics of numeric variables and mean difference of serum 25(OH)D level at baseline with month 3 and month 6 between the two treatment groups. Chi-square test was used for comparison of qualitative variables between the two groups. Using Kolmogorov–Smirnov test, we confirmed normality of distribution of 25(OH)D level. Therefore, to compare mean serum 25(OH)D levels within each group, at first and last measurement, paired t-test was used. Repeated measurement analysis of variance (anova) was performed to compare the changes in serum 25(OH)D level in patients of two groups. $P < 0{\cdot}05$ was considered statistically significant. All the statistics were calculated using spss version 16·0 (SPSS Inc., Chicago, IL, USA).

Results

Of 150 patients assessed primarily for eligibility, 92 patients were included; 46 patients in each group. During the first 3 months of study, seven patients of group 1 and six patients of group 2 were lost to follow up. In the next 3 months of the study, some other cases were excluded from both groups. At the end, 61 patients completed the study; but 79 patients were included in final per-protocol analysis using LOCF method (Fig. 1). The percentage of dropouts between the two groups was similar. There was no statistically significant difference at baseline between who completed the study (*n* = 61) and who did not (*n* = 31). Baseline characteristics of the patients were similar between the treatment groups (). Compliance of the groups in drug usage was acceptable (>90%) and comparable.

Table 1. Baseline characteristics of patients

Variable	**Group 1 (injection) (*n* = 39)**	**Group 2 (oral) (*n* = 40)**	***P***
Age (year)	49·6 ± 1·5	48·1 ± 1·2	NS*
BMI (kg/m^2)	26·9 ± 0·6	27·6 ± 0·8	NS
Women (*n*)	30	33	NS
Baseline serum vitamin D level (nmol/l)	36·3 ± 2·3	37·5 ± 2·5	NS
Daily sun exposure (min)	33·8 ± 3·1	41·2 ± 4·5	NS
<2 times/week fish consumption (*n*)	30	34	NS
<2 times/week eggs consumption (*n*)	29	27	NS

Data denoted as Mean ± SEM, except for numbers.
*NS, not significant.

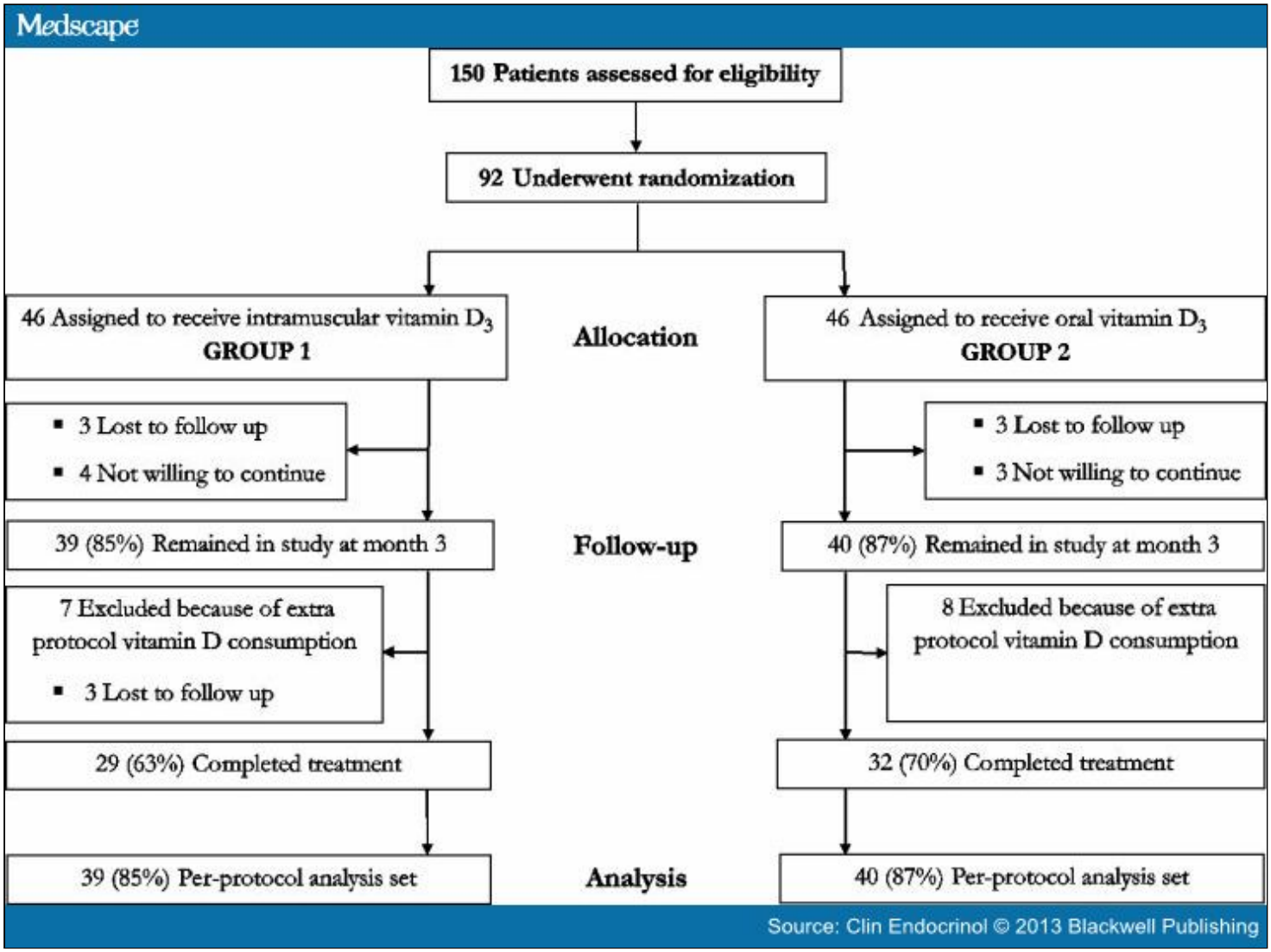

Figure 1.

Flow diagram of enrolment, randomization and follow-up.

Serum 25(OH)D Responses to Treatment

The range of serum 25(OH)D levels throughout the entire study period is shown in Fig. 2a. Mean serum 25(OH)D in group 1 was 36·3 ± 2·2 nmol/l at baseline, which raised to 95 ± 9·2 and 88·4 ± 7·8 nmol/l after 3 and 6 months of intervention, respectively ($P < 0·001$). Mean serum 25(OH)D in group 2 was 37·5 ± 2·4 nmol/l at baseline, which increased to 127 ± 11·4 and 99·8 ± 7 nmol/l after 3 and 6 months of treatment, respectively ($P < 0·001$) []. Using repeated measurement of anova, it appeared to be a marginally significant rise in serum 25(OH)D in favour of the oral route during time (Data not shown, $P = 0·07$). Figure 2b shows delta changes in serum 25(OH)D levels; the mean change in serum 25(OH)D level from baseline at month 3 was significantly higher in oral than injection group (90 ± 11·2 and 58·8 ± 8·9 nmol/l respectively; $P = 0·03$). There was no significant difference between the delta changes in 25(OH)D level achieved in injection and oral groups at 6th month (52·1 ± 7·6 and 62·2 ± 6·7 nmol/l respectively; $P = 0·32$).

Table 2. Mean serum 25(OH)D level after 3 and 6 months in two groups

Variable	Group 1 (injection) ($n = 39$)	Group 2 (oral) ($n = 40$)	P*
Baseline serum25(OH)D (nmol/l)	36·3 ± 2·2	37·5 ± 2·4	0·7
Serum 25(OH)D after 3 months (nmol/l)	95 ± 9·2	127·5 ± 11·4	0·03

Serum 25(OH)D after 6 months (nmol/l)	88·4 ± 7·8	99·8 ± 7	0·28
P †	<0·001	<0·001	▫

Values denote Mean ± SEM.
*Injection *vs* oral, comparison by independent *t*-test.
†Within group's comparison by repeated measurements anova. Last observation carried forward method was used for missing data at 6th month.

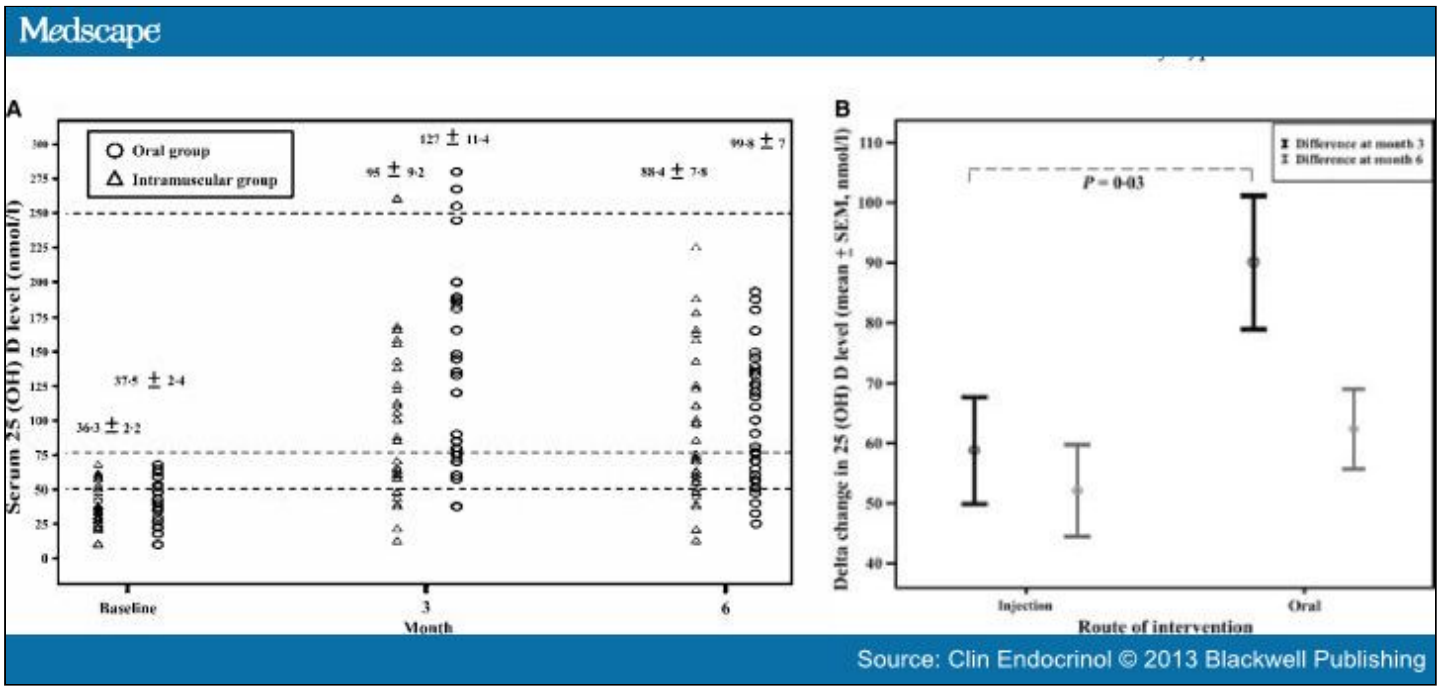

Figure 2.

Comparison of serum 25(OH)D levels in the study patients. (a) Distribution of serum 25(OH)D levels at baseline, and after 3 and 6 months intervention in two groups. Numbers denote mean± SEM. The dashed lines represent the threshold levels for vitamin D insufficiency, and adequacy range settled at 50, 75 and 250 nmol/l. (b) Observed delta changes in serum 25(OH)D levels after 3- and 6-month treatment in two groups. Last observation carried forward method was used for missing data at 6th month.

The percentage of patients receiving serum 25(OH)D above cut-off points of > 50 and > 75 nmol/l is shown in . At 3 months, serum 25(OH)D level increased to ≥ 75 nmol/l in 51·3 and 65% in group 1 and group 2, respectively (*P* = 0·21); and 3 months later, it remained ≥ 75 nmol/l in 43·6 and 65·6% of cases in group 1 and group 2, respectively (*P* = 0·06).

Table 3. Percentage of participants with serum 25(OH) D levels above a certain cut-off point after 3 and 6 months of treatment in two groups

Variable	**Group 1 (injection) (*n* = 39)**	**Group 2 (oral) (*n* = 40)**	***P****
Serum 25(OH)D ≥ 75 nmol/l			
3 months	51·3 (20)	65 (26)	0·21
6 months	43·6 (17)	65 (26)	0·06
Serum 25(OH)D ≥ 50 nmol/l			
3 months	76·9 (30)	92·5 (37)	0·06
6 months	82·1 (32)	90 (36)	0·30
*Injection *vs* oral, comparison by Pearson chi-square test. Data denote *Percentage* (*number*). Last observation carried forward method was used for missing data at 6th month.			

Three months after treatment, serum 25(OH)D > 250 nmol/l (range: 255–280 nmol/l) was detected in 2 and 4 patients of injection and oral group, respectively (P = 0·7); all normalized to < 250 nmol/l at 6 months (data not shown). None of them had hypercalcaemia or hypercalciuria at 3 months.

No adverse reaction including discomfort, erythema, cellulitis, swelling, skin atrophy, fever or abscess formation was reported during the study in either treatment groups.

Discussion

The present study demonstrates the considerable efficacy and safety of two different oral and injectable regimens utilizing a total dose of 300 000-IU vitamin D_3 in treating hypovitaminosis D. In both groups, at least 75% of cases achieved 25(OH)D levels of > 50 nmol/l, and about 50% achieved levels of > 75 nmol/l at 3 and 6 month of intervention. The delta change in 25(OH)D level was significantly higher in oral route at 3 months, and there was a marginally significant trend in rising 25(OH)D level in favour of that route during the entire follow-up time. About 8% (6 of total 79) of treated cases developed 25(OH)D level of > 250 nmol/l at 3 months intervention, which made us worry about potential toxicity. The highest 25(OH)D level was 280 nmol/l and under the 375 nmol/l that is considered to be potentially toxic;[25] it was found in a case from injection group with a near normal pretreatment level of 73 nmol/l. Too much vitamin D may cause acute toxicity presenting with symptoms of hypercalcaemia, or hypercalciuria. However, none of our patients with levels of > 250 nmol/l showed any symptoms of toxicity or developed hypercalcaemia or hypercalciuria. At finish point of our study, no cases with 25(OH)D level of > 250 nmol/l were found.

Vitamin D deficiency is rather widespread and common in different parts of the world, but there is no consensus in the ways of its treatment. The results of studies on treatment with different low or high doses of vitamin D_3 are inconsistent. Papaioannou *et al*.[26] showed that 90 000 IU D_3 had marginally better results than 190 000 IU vitamin D in a 3 months period, both in the level of 25(OH)D achieved and also in the proportion of subjects reached vitamin D adequacy. Considering all controversies, one recommendation with proven safety and efficacy has been based on oral use of 50 000 IU vitamin D_2 or D_3 once weekly for 8 weeks and then to continue daily dose of 1500–2000 IU.[25, 27] It worth to pay attention to effect of body weight on dose response curve; based on findings of Wortsman *et al*., larger doses of vitamin D may be required in treating vitamin D-deficient obese persons compared with nonobese ones, possibly due to decreased vitamin D bioavailability.[25, 28] It is obvious that success of such a regimen depends at least in a major part, to patient adherence with getting two different drug dosage and time course. In today's busy life, it is well possible that getting mistakenly the high dose of the above-mentioned regimen in weekly manner for a long time would result in vitamin D toxicity. Any simple dosing regimen may allow convenient outpatient management with improvement in compliance. There is still parenteral high dose form of vitamin D available in some parts of the world. In addition to the notable concerns about compliance and toxicity, longer duration of oral treatments, presence of malabsorption syndromes and also cost of treatment are some important issues in selecting the way of treating vitamin D deficiency. On the other hand, there are some warnings in the literature about using injection of high-dose vitamin D, both as far efficacy and toxicity.

The majority of the published data before the year 2000 have not followed up the cases for enough time period, have been performed in small sample size of a certain population (old institutionalized) with narrow age range and, more importantly, have used the previous definition of sufficiency with lower cut-off point of normality which is considered as definite hypovitaminosis in the new era of vitamin D understanding.[29] There are some recent studies targeting vitamin D therapy with pharmacologic doses of the vitamin. Diamond *et al*.[30] showed that an annual intramuscular injection of 600 000 IU cholecalciferol was safe and effective for treatment for vitamin D deficiency in 50 Australians. Another group from Australia compared oral continuous low-dose *vs* short-term high-dose vitamin D in treating vitamin D-deficient cases; one group received 500 000 IU oral vitamin D during 10 days, and the other group received 150 000 IU vitamin D orally during 3 months on a daily manner. Their study proved both regimens to be effective in increasing serum 25(OH)D to within normal range.[31] These two recent studies are similar to our study in age range and number of cases, in the criteria used for insufficiency, and also in proven efficacy and safety of megadoses used. Their main findings were also similar to our results in each of our oral or injectable arms of trial; of coarse their study protocol and their vitamin D dosage were different from our study. Giusti *et al*. used two different D_3 dosages in treating 60 elderly vitamin D-deficient women in a 6-month follow-up study. One group received total 600 000 IU D_3 in two equal doses, one at baseline and the second at 3 months; the other group was put on daily dose of 1000 IU D_3. The findings of high-dose arm at 6th month was rather similar to ours in the delta change in 25(OH)D achieved and also in the rate of cases reached vitamin D adequacy, of course with getting two times more vitamin D_3.[32] Study of Hashemipour *et al*.[33] raised some concerns about

using megadoses of intramuscular vitamin D. It showed that vitamin D injection especially at doses higher than 300 000 IU may be associated with hypervitaminosis D. Their study included volunteers with near normal baseline 25(OH)D level that is quite different with our cases.

To our knowledge, there are very few published studies comparing the same dose of different routes of vitamin D_2 or D_3 in treating hypovitaminosis D. The old one published in 1981, used 100 000 IU D_2 orally or intramuscularly to treat a group of Asians with serum 25(OH)D of < 12·5 nmol/l.[34] Comparable to our results, it showed both regimens were similarly effective in raising serum 25(OH)D level at finish point of the study. They also showed superiority of oral to injection route at 1-month treatment, the finding we found at 3rd month of our study. In contrast to ours, based on current definition of 25(OH)D normality, none of their patients reached normal values of 25(OH)D. This may be explained by their very low baseline serum 25(OH)D level and also by the smaller dose of vitamin D used. The study by Leventis *et al*. tried to compare oral and intramuscular regimens with same dose of 300 000 IU in treating cases with 25(OH)D of < 40 nmol/l. Similar to ours, their study concluded that at 3 months the oral route was superior, and the two regimens were equal at 6 months. However, They had used two different pharmaceutical agents (oral D_3*vs* injectable D_2), and also their two groups were significantly different in age and baseline 25(OH)D.[35] We have recently presented the results of a preliminary study comparing two routes of treating hypovitaminosis D, which showed the effectiveness of both methods, oral and intramuscular. Although the oral route was found as superior one at 3-month intervention, it should be emphasized that vitamin D_3 dosage used for treatment for vitamin D deficiency was different between the groups; 450 000 IU was used for oral group, while the patients in intramuscular arm received 300 000 IU.[36] Considering the methodological problems of that experience, we designed the present study with an equal dosage plan, and also with a longer follow-up period, to get more accurate and reliable results.

The current study carries some limitations. First, this was an open study with no blindness. In our opinion, it was not ethical to perform painful placebo injection only to achieve blindness. Second, we had some dropouts because of excluding patients who had any extra-protocol vitamin D usage. Third, using questionnaire for assessing compliance of oral group could be affected by recall bias; and fourth, if we had measured serum calcium, phosphorus and PTH in all cases, the study results might have answered to some other possible unclear points in this field.

In conclusion, by using new definition of serum 25(OH)D normality, our study provides preliminary evidence that the same high doses of vitamin D_3 are effective, practical and safe, either orally or parenterally in treating vitamin D deficiency. However, it should be emphasized that even with the high dose of vitamin D we used; about half of cases did not achieve the adequate level. Although we revealed superiority of oral route, at least in the early short time of our study, facing the widespread worldwide pandemic of hypovitaminosis D, physicians should firstly be aware of its presence and secondly may choose the way of treatment based on patient's wish, compliance and availability of various forms of the drug in their regions. Further investigations with longer follow-up in future will help to definitely recommend such a therapeutic approach.

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