Research Paper

Comparison of Serum 25-Hydroxyvitamin D Levels After A Single Oral Dose of Vitamin D3 Formulations in Mild Vitamin D3 Deficiency

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Abstract

Objective: To compare the levels of serum 25 Hydroxyvitamin D levels after a single large oral dose (60,000 IU) of different vitamin D3 formulations. **Materials and Methods:** Ninety-one volunteers with mild vitamin D deficiency (18–29 ng/ml) were selected and randomly assigned to three parallel groups. Groups-I received liquid, Group-II received sachet, and Group-III received tablet formulation of cholecalciferol as a single dose of 60,000 IU orally after 8–10 h of overnight fasting. Serum 25(OH) D concentrations were measured at baseline, 24 h, 7 days, and 14 days after drug administration. Various hematology and biochemical parameters were also assessed for baseline safety evaluation. **Results:** Baseline serum 25(OH) D concentrations in Groups I (liquid), II (sachet), and III (tablet) was 24.75 ± 4.77 ng/mL, 23.25 ± 4.15 ng/mL, and 23.18 ± 5.52 ng/mL, respectively. After supplemented with three formulations, only tablet group after 24 h showed increase in serum 25-OH-D concentration of 8.07 units from its baseline. Whereas after 7th day, no significant difference in absorption was observed but after 14th day, all three groups showed increase in serum 25-OH-D concentration, in which tablet group (50.10 ± 94.99 ng/ml) showed highest increase in absorption (26.92 units) from their baseline values. During intergroup comparison between three formulations at the time of investigation, only liquid group after 24 h showed increased serum concentration by *P* values (0.03, 0.02) as compared to sachet and tablet group. However, After 7th and 14th day, there was no statistically difference was observed between three groups. **Conclusion:** Single oral dose of 60,000 IU dose of vitamin D liquid formulation has higher absorption value as after 24 h and tablet formulation showed higher absorption after 7th days. In emergency paucity of vitamin D, these observations findings can have critical conclusions to state the suitable dietary formulation of vitamin D.

Keywords: Dosage form, mild vitamin D deficiency, single dose, vitamin D3

Introduction

Vitamin D insufficiency has been identified as a more frequent worldwide condition. Vitamin D is the most commonly prescribed oral nutraceutical in countries like India and other South Asian countries.[1,2] Vitamin D gets synthesized in the skin on exposure to sunlight and comes from diet also.[3] Serum calcidiol is a functional biomarker of vitamin D status. Vitamin D deficiency was formerly linked to osteomalacia and osteoporosis or osteopenia. Many ailments, including hypertension, diabetes,S asthma, colon cancer, multiple sclerosis, atherosclerosis, various neurological disorders,

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neurodermatitis, psoriasis, collagenosis, and immune system infection, have now been related to vitamin D insufficiency.[4]

Vitamin D is usually taken orally or as an intramuscular injection. Vitamin D injections, on the other hand, can cause painful granuloma, hemorrhage, and infections at the injection site, delaying absorption even further.[5]

Oral route has been considered as most obvious route of administration due to high patient compliance. In view of patient compliance and established therapeutic roles of vitamin D in literature, it would be crucial, if bioavailability of oral formulations of vitamin D could be increased independent of meals.[6] Vitamin D bioavailability remarkably varies with nature and type of formulation and the excipient used.[7] It has a long half-life and requires less dosing frequency, and thus provides high patient compliance. The potency of oral absorption of the current vitamin D is nearly 50%.[8]

Despite the fact that multiple research studies have been conducted on the effects of various methods of vitamin D supplementation, none of them have compared the single large dose effect of three formulations on short term (24 h, 7 days, and 14 days) absorption in urgency of overcoming mild vitamin D depletion. Furthermore, single large dose of vitamin D3 is useful for patient convenience and long-term adherence.

Materials and Methods

This parallel arm randomized study included 93 participants with mild vitamin D deficiency of age between 18 to 50 years in Physical Medicine and Rehabilitation OPD, Dr. Ram Manohar Lohia Hospital, New Delhi, India. Thirty-one people were divided into three groups randomly: liquid (Group 1), sachet (Group 2), and tablet (Group 3). Participants with a serum 25(OH)D level ≥22 and ≤29 ng/mL at screening were enrolled and randomized for the study. At screening, participant with known vitamin D3-related hypersensitivity, any previous history of taking medication including herbal remedies of containing active vitamin D3 compounds or a high dose of vitamin D3 (>5000 IU) within 30 days before study were not considered for the randomization. Participants with a serum 25(OH) D level <22 and >29 ng/mL at screening, participants with major diseases during the 60 days before screening and participants with inconsistent diet scheduled during the month preceding the study (i.e., fasting and high-protein diets), and travel outside the Delhi (study site) during the 14 days of study period were excluded from the study [Figure 1].

After being checked for mild vitamin D deficiency in the PMR OPD in research facility, all participants were asked to come for group randomization on the next day at 8:00 a.m after overnight fasting, in the blood sample for, 25(OH) D, total calcium, urea, creatinine, uric acid, Serum glutamic pyruvic transaminase, Serum glutamic oxaloacetic transaminase, bilirubin, and complete hemogram with erythrocyte sedimentation rate (ESR) were taken. After that, each participant was given 60,000 IU of cholecalciferol in the form of a sachet or tablet or

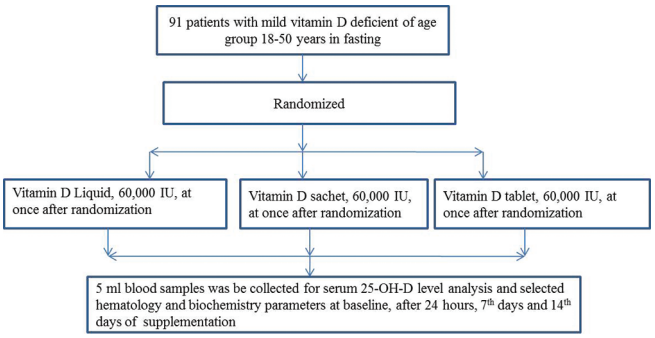

Figure 1: Absorption comparison study protocol for single oral large dose of Liquid, Sachet and tablet of Vitamin D formulation

liquid by the oral route. For the next 2 weeks, the participants were told to avoid fortified cereals, orange juice, egg yolk, mushroom, ricotta cheese, fatty fish, cod liver oil, and fish eggs, as well as changes in their usual dietary habits. They were also advised to keep a food diary. All participants in each groups were advised similar dietary and lifestyle. Overnight fasting blood samples were taken after 24 h, 7 days, and 14 days for the measurement of 25(OH) D. In the same run, all samples were analyzed: using Chemiluminescence assay for 25(OH) D (LIAISON, DiaSorin Inc., Stillwater/MN, USA, intraassay coefficient of variation = 5.5%).

The number of participants was estimated using the statistical software SPSS version 17 (Integrated Student Version 17.0, for window and mac, USA). The data were presented as counts (%) or mean ± standard deviation/median (Min - Max). For categorical variables, the Chi-square test was used, and for continuous variables, the t-test and analysis of variance was used to compare the baseline values of the groups. The results were expressed as a percentage difference (95% confidence interval). Statistical significance was defined as a $P < 0.05$. The study was approved by the Institutional ethics committees of Atal Bihari Vajpayi Institute of Medical Sciences, Dr. Ram Manohar Lohia Hospital (no.-216 (35/2017)/0IEC/PGIMER/RMLH) 744/18) New Delhi, India and registered by Clinical Trial Registry-India (CTRI/2019/06/026620). Patient screening, enrolment, randomization, and treatment begin from June 2018 to November 2019 when a projected interim analysis was executed. After receiving written informed consent, participants were enrolled in the study.

Results

Groups I (liquid), II (sachet), and III (tablet) had baseline serum 25(OH) D concentrations of 24.75 ± 4.77 ng/mL, 23.25 ± 4.15 ng/mL, and 23.18 ± 5.52 ng/mL, respectively [Table 1]. After single oral, one-time large dose (60,000 IU) treatment with liquid, sachet (granules), and tablet, after 24 h, only tablet group shows highest increase in 25-OH-D concentration of 8.07 units to liquid and sachet from its baseline [Table 1]. Whereas after 7th day, there was no noticeable absorption difference observed in between three groups when compared to their baseline and after 24 h values [Table 1]. However,

Table 1: Serum 25-OH-D absorption levels at baseline, after 24 h, at 7th day and at after 14th day between liquid, sachet and tablet group

Sampling time	Group I (liquid)	Group II (sachet)	Group III (tablet)
Baseline sample value	24.75±4.77	23.25±4.15	23.18±5.52
24 h	25.23±7.78	25.23±7.78*	31.25±11.79*
7th day	27.16±8.44	27.16±8.44	31.63±11.15
14th day	42.20±73.92	42.20±73.92	50.10±94.99

Values (ng/mL) represent mean±SD. n=31 in each group. *P<0.05 when compared to group I.

after 14th day of dose treatment, all three groups showed increase in serum 25-OH-D concentration: 42.20 ± 73.92 ng/ml in liquid group, 42.20 ± 73.92 ng/ml in sachet group, and 50.10 ± 94.99 ng/ml in tablet group, which showed highly increase in tablet group by 26.92 units from its baseline values [Table 1].

After 24 h, the intergroup comparative analysis of vitamin D absorption in fasting, group comparison between liquid, sachet, and tablet revealed that individuals receiving vitamin D in liquid dosage form have a higher rate of vitamin D absorption by P values (0.03, 0.02) than those receiving vitamin D in sachet and tablet form [Table 1]. When compared to each other; however, serum 25(OH) D mean concentrations were higher on the 7th day in those who got all three dosage forms, although not statistically significant [Table 1]. Absorption comparisons after 14 days of dosage administration revealed no statistically significant differences between the liquid, sachet, and tablet groups [Table 1].

Differences among certain hematological and biochemical parameters have been observed between groups. The liquid group had a higher alkaline phosphatase (mean difference 24.98 ± 9.07, P = 0.006) when compared to sachet group. The level of hemoglobin in liquid group is significantly different from that of sachet (mean difference –0.122 ± 0.44, P = 0.01) and tablet group (mean difference –1.57 ± 0.44, P = 0.001). Also the level of ESR in liquid group is significantly different from that of tablet group (mean difference 17.32 ± 1.05, P = 0.03) .

Discussion

Insufficiency of vitamin D largely affects the physical and mental health of an individual. It is analyzed that India alone has >90% of reported healthy individuals bearing 25-(OH) D deficiencies.[9] As per the available reports, a lowest of 800–1000 IU of vitamin D3 is required on daily basis to attain 32 ng/ml mean serum of 25(OH) D levels in and the current recommended guideline in India for amelioration of vitamin D level in an individual is 60,000 IU vitamin D weekly for 8 weeks.[10] Many authors take this value in relation to the optimal calcium absorptive performance,[11] as the verge of appropriate vitamin D3 status.[12-14] However, research on the appropriate dose, dosage form, and dosing intervals for achieving and maintaining these levels, as well as preventing hypovitaminosis D deficiency in general population is deficient. Several studies have found that supplementing with substantial dosages of vitamin D is safe and effective.[15-18] A single high dose of cholecalciferol has been recommended to help patients with deficiency of severe vitamin D and quickly boost their serum 25(OH) D levels. Furthermore, greater cholecalciferol intermittent dosages were advised to improve adherence to medication.[12,17,19] The highest therapeutic dose of acceptable vitamin D daily intake has been revaluated, with a trend to shift to 2000 IU per day according to new guidelines.[20]

Oral dosage forms such as tablet, capsule, and sachet have different absorption rates. The efficiency of oral absorption of conventional vitamin D3 is approximately 50%.[21] In general, the availability for absorption of a drug is more in oral solutions compared to the capsule and tablet, respectively.[22]

Despite the availability of vitamin D3 oral solution in the market, none of the studies has been conducted which compares the oral vitamin D formulations, i.e., sachet, solution, and tablets in serum blood levels after getting orally. The main objective of this study was to compare the serum 25-(OH) D levels in blood of different oral vitamin D3 formulations.

Hence, the current study was designed to investigate the effect of three different oral single high dosage of vitamin D (600,000 IU) in liquid, sachet, and tablet forms on serum blood levels of 25(OH) D in participants with mild vitamin D insufficiency.

Previous study of oral dosage form on absorption rate revealed that oral solution has greater absorption rate compared to the other dosage forms. The process of absorption with different formulations gets reduced as in the sequence given: solution > suspension > powder based capsule > compressed tablet > coated tablet.[22]

In the present study, we found similar result of absorption of vitamin D from different oral dosage forms from previous one after 24 h. The comparative analysis of 25-(OH) D absorption between liquid, sachet, and table in fasting condition revealed significant increase in serum concentration of vitamin D levels in liquid dosage form followed by sachet and tablet dosage forms.

Our observed result suggests that a 60K IU of vitamin D dose administered orally can rapidly raise serum 25-(OH) D levels in patients with mild vitamin D insufficiency. The serum level of25-(OH) D was achieved as early as 24 h following vitamin D treatment, presumably due to the utilization of a substantially greater dose than previously reported.[6] Significant increment in serum 25(OH) D levels was also achieved at 3 days when 300,000 IU of vitamin D was given to older age participants.[17] However, we noticed that highest value reached by an individual participant in the present study was 59 ng/mL, well beneath to the generally recognized toxic therapeutic levels of 200 ng/mL.[23] This result was possibly due to the low pretreatment vitamin D status of study participants. In addition, vitamin D3 levels continued substantially greater

than baseline data up to 14 days for all 91 participants followed up to 14 days. At the end stage of our study, we observed that 46 patients still had vitamin D levels over 42 ng/mL.

In total, our findings show that a greater oral dose of vitamin D3 produces and retains appropriate levels of 25-(OH) D in persons with mild vitamin D insufficiency for up to 14 days. These findings are consistent with data obtained in elderly participants and are likely to be focused on fast absorption and alteration of the oral dose of vitamin D3 to the 25-hydroxy metabolite. Heaney *et al*. in his research[18] have shown that cholecalciferol intake initiates a biphasic result, with a sudden boost of serum 25(OH) D at lesser serum vitamin D3 level and a lower response at greater levels. We may therefore, consider that the rise in vitamin D levels after vitamin D dosing could possibly be attributed to the low baseline vitamin D3 value.

Ilahi *et al.* found a serum 25-(OH) D level 7 days following a one-time oral intake of 100,000 IU of vitamin D, although other researchers found comparable values 1 month later in participants administered with 500,000 IU of oral cholecalciferol. The vitamin D levels in the beginning were 20 ng/mL or less.[19,24] Still no data for 3–6 days following vitamin D infusion were available. On the other side, a single time oral 60,000 IU dose of cholecalciferol may raise serum 25(OH) D concentrations above the threshold of adequacy only at 120 days in patients with vitamin D inadequacy.[25] This result affirms that oral intake is a beneficial possible route for vitamin D dosing when faced with physiological skin development. This is also confirmed by our groups' previous findings showing that vitamin D in dose of 60,000 IU given orally rather than intramuscularly can piercingly and adequately raises 25(OH) D levels in mild vitamin D inadequate participants.[17] That study analysis, the maximum vitamin D level was increased at 7 days, and it retained significantly greater than basal value till 7 days. We may deduce that oral cholecalciferol 60,000 IU dose causes a large increase in 25-(OH) D serum levels while retained appropriate vitamin D level for till 14 days. Furthermore, younger and less deficient participants took a one-time oral dose of 60,000 IU which resulted in a faster rise in serum 25(OH) D levels and stable vitamin D value for up to 14 days.

The considerable rise in serum calcium amount 3 days after vitamin D intake could be owing to raise of calcium absorption as a result of the increased 25(OH) D values.[11]

In fasting group, liquid and sachet showed better absorption of vitamin D when compared to tablet dosage form when compared to other dosage form at 24 h.

Our study also favors the statement that absorption rate of oral liquid, sachet, and tablet dosage form of vitamin D seems to be unpredictable when taken in fasting. Interestingly, recent studies found divergent observations from our findings and advised that supplementation of vitamin D with the main course of the day enhanced vitamin D absorption.

Raimundo *et al.* 2014, in a clinical study on 64 participants revealed that a one-time oral powder capsule of 50,000 IU vitamin D3 dose given with fatty meal raises the 25(OH) D3 serum level after 14 days.[26]

However, bioavailability is not only related to the active molecules but it is also depends on formulations and excipients used in the increase of the pharmaceutical dosages form of vitamin D3. In the current study, variable rate of vitamin D absorption from different dosage form may be attributed to the use of different excipients in the respective formulations.

In nutshell, all three formulations (liquid, sachet, and tablet) of vitamin D caused significant increase in 25(OH) D3 serum concentrations at 24 h, 7th day 14 days, respectively, when compared to baseline concentrations. However, we do not find any significant effects of fasting on 25(OH) D3 serum concentrations at 7th day 14th days in any of the groups received liquid, sachet, and tablet formulations of vitamin D.

Conclusion

Our findings have some clinical implications that have been noted. The potency of a relatively large dose of cholecalciferol given orally in patients with a high risk of vitamin D insufficiency and a high fracture risk is a remarkable result. It is also a good idea to prevent hypocalcemia in vitamin D deficient patients before starting IV bisphosphonate medication. Furthermore, we believe that a single cholecalciferol loading dose is associated with good adherence to medication, especially in young people with vitamin D insufficiency.

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Conflicts of interest

There are no conflicts of interest.

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