Efficacy and safety of various oral regimens (three oral doses) and schedules (daily vs. monthly) of cholecalciferol in north Indian adults with low vitamin D status: Evidence from a randomized controlled trial

Mohd Ashraf Ganie MD, DM[1, 2] Professor, Shivani Sidana MD, DM[1] Senior Resident, Mohammad Salem Baba MD, DM[1] Senior Resident, Tajali Sahar PhD[2] Scientist-B, Aafia Rashid PhD[2] Scientist-B, Mir Mahmood Asrar PhD[2], Scientist-I, R. K Marwaha MD, DNB[3] Consultant, Ishfaq Ahmad Wani PhD[2] Scientist-B, Riyaz A Misgar MD, DM[1], Associate Professor, Imtiyaz Ahmad Wani PhD[2] Scientist-B

[1]Departments of Endocrinology, Srinagar and ILSI India, New Delhi India

[2]Clinical Research, Srinagar and ILSI India, New Delhi India

[3]Sheri-Kashmir Institute of Medical Sciences, Srinagar and ILSI India, New Delhi India.

Short Title: Optimal Vitamin D dosage among adults

Corresponding author: Prof. Mohd Ashraf Ganie (MD, DM), Head Department of Clinical Research and Professor of Endocrinology, R.NO- P118, Sheri-Kashmir Institute of Medical Sciences, Srinagar, India, Contact no-+91-194-2401315 (o) ;9419041546 (M), Email: ashraf.endo@gmail.com

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Abstract:

Vitamin D (VD) deficiency [serum 25(OH)D concentration of <20 ng/ml], in endemic proportions, demands a supplementation strategy with optimal dosing regimens. A randomised parallel-group, active-controlled trial was conducted among apparently healthy, VD deficient subjects, age 18-60 years who received 600 IU/day (Group A), 1000 IU/day (Group B), 2000 IU/day (Group C) and 60,000 IU/month (Group D) of oral cholecalciferol. The intervention was carried in two phases (I&II) of 12 weeks each, with same dose, separated by a washout phase of 12 weeks. Serum 25(OH)D, iPTH, calcium (Ca), phosphorous (PO4), alkaline phosphatase (ALP), spot urine calcium/creatinine (Ca/Cr) was measured at baseline, 12, 24 and 36 weeks following the intervention and adverse events were recorded at each occurrence and at 12, 24 and 36 weeks. A statistically significant time-group interaction was found in serum 25(OH)D concentration ($P<0.05$). Serum 25(OH)D concentration increased significantly from baseline to 12 weeks ($P<0.05$) in all the groups with no change at 24 weeks but further increase at 36 weeks ($P<0.05$). At the end of study, group C had maximum increment in serum 25(OH)D concentration while as groups C and D (95%, and 90%) had higher proportion of subjects VD sufficient than groups A and B (65% and 78%) (Table 3) ($P<0.05$). No significant time-dose interactions were observed in serum iPTH, Ca, PO4 and ALP or Urine Ca/Cr ratio. Three subjects (two in group C and one in group D) developed transient hypercalciuria. Supplementation with daily 2000IU or monthly 60,000IU oral cholecalciferol among adults seems optimal and safe.

Key words: Vitamin D deficiency**,** Tropical regions, 25 (OH)D**,** cholecalciferol, iPTH

Abbreviations: iPTH -Intact parathyroid hormone, VD- vitamin D, VDD- vitamin D deficiency, HOMA-IR- homeostatic assessment model for insulin resistance, IOM- Institute of medicine, ES- endocrine society, ICMR- Indian council of medical research, NCD- Noncommunicable diseases, DM- diabetes mellitus, CVD- cardiovascular diseases, ALT- alanine transferase, AST- aspartate transferase, ALP- alkaline phosphatase, BMI- body mass index, LDL-low density lipoprotein, HDL- high density lipoprotein,

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Introduction

Despite the endogenous synthesizing capability of individuals, vitamin D (VD) deficiency is widespread globally [1]. India is a tropical country with plenty of sunshine and the prevalence of VD deficiency, defined as serum 25(OH)D concentrations of <20 ng/ml ranges from 70-100%. [2–4], This magnitude of VD deficiency in India mandates food fortification, which is as yet not in practice [4] However, for any effective implementation strategy, there is a need to assess the dose-response relationship between supplemented VD dose and serum 25(OH)D concentrations.

The recommended daily intake of oral VD in populations at risk of VD deficiency to achieve a serum 25(OH)D concentration of more than 20 ng/ml by the Indian Council of Medical Research (ICMR) [5], Indian Academy of Paediatrics (IAP)[6] and National Academy of Medicine (NAM) [7] is 600 IU/day for ages ranging from 1-75 years. In contrast, the Endocrine Society (ES) recommends daily intake of 1000 IU/day for 1-18 years old and 1500 – 2000 IU for all men and women aged above 18 years including pregnant/lactating women, to keep serum 25(OH)D concentrations of >30 ng/ml considered sufficient for overall good health [8]. The dilemma, whether serum concentration of 25(OH)D of >30 ng/ml translates into non-skeletal benefits such as prevention of infections, non-communicable disease (NCDs) like insulin resistance (IR), diabetes mellitus (DM), cardiovascular diseases (CVD), psychiatric morbidities, cancer etc. [9–11] due to its pleiotropic effects, continues to persist. The guidelines which suggest to raise serum 25(OH)D >20 ng/ml for skeletal benefits are not backed by high quality evidence and mostly involve studies in western populations [7,12].

In India, there is a lack of clear-cut data on the optimal dose/schedule of VD supplementation to achieve serum 25(OH)D >20 ng/ml, owing to heterogeneity of the studies about age, duration (8 weeks to 1 year), doses (200 to 60,000 IU), sample size, level of serum 25(OH)D considered sufficient and populations studied [13–16]. Hence, we undertook this randomized study with to evaluate the comparative effectiveness of three different daily dosage regimens of oral cholecalciferol supplementation among themselves and with once-monthly schedule. The main objective was to find appropriate oral VD dose required to achieve VD sufficiency [serum 25(OH)D >20 ng/ml] at the end of 36 weeks in VD deficient Indian adults.

Material and Methods

Trial Design and Oversight

A randomised parallel group, active controlled trial was conducted at the department of endocrinology at a tertiary care institution of northern India (Latitude: 32°44'N, Longitude: 74°54'E) from June 2019 to December 2020, however, subjects were recruited and first dose administered within 2 weeks from 1st June to 31st July in 2019 and 1st April to 30th May 2020. The study was conducted according to 1975 declaration of Helsinki. The trial was approved by the Institutional Ethics Committee and registered with Clinical Trial Registry of India (CTRI/2019/01/016855).

Participants

The study participants were invited through advertisements in pamphlets, print and electronic media, detailing the standard operating procedures of the study.

Inclusion & Exclusion Criteria

All healthy subjects (both males and females) in the age group of 18-60 years who signed an informed written consent were included. The enrolled participants were subjected to a questionnaire-based interview. Age, education level, sun exposure (duration of exposure and body surface area exposed to sunshine based on rule of nine formula)[17], dietary consumption, history of any systemic disorders, medications, health supplements etc. were recorded at screening. Participants with history of any chronic illness (Diabetes mellitus, hypertension, stroke, malignancy, kidney, liver, heart bone or endocrine dysfunction etc.) or drug intake in previous 2 months likely to affect calcium and VD metabolism were excluded. Other exclusion criteria included pregnancy, miscarriage, or lactation. The participants having serum 25(OH)D concentrations above 20 ng/mL were excluded from the study.

Randomization, Allocation concealment and Blinding

The subjects were randomized into one of the four groups [A: 600 IU/day; B: 1000 IU/day; C: 2000 IU/day; D: 60,000 IU/month] in 1:1:1:1 ratio using computer generated random number sequence. Subjects were provided an opaque and sealed envelope containing the random sequence number for the intervention. The envelopes were opened in a numerical sequence, as each new person entered into the trial. The trial was partially blinded. All the subjects and the investigators were blinded to three arms (daily doses of cholecalciferol),

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however, the monthly dosing schedule arm was open label. All subjects received the drug in the form of odorless, amber colored capsules packed as 30 capsules in coded unlabeled bottles with the exception that the subjects in the 4th arm received the drug as a single monthly capsule with similar attributes. The random sequence was deposited with central pharmacy until all the data was analyzed. The cholecalciferol capsules were manufactured by a standard protocol by pharmaceutical company (USV Private Ltd, Mumbai, India) and the company had no role in data collection or randomization. VD content of capsules was analyzed independently by another laboratory to contain cholecalciferol within 90-110 % of labelled amount. The trial schedules as well as the results of the randomization are shown in flow chart (Figure 1).

Treatments and Dosing Schedule: The included subjects were supplemented with oral cholecalciferol doses as following: Group A: 600 IU/day; Group B: 1000 IU/day; Group C: 2000 IU/day; Group D: 60,000 IU/month initially for 12 weeks (phase I). These subjects were then kept off supplementation for the next 12 weeks (13-24 weeks; washout phase). Subsequently, the subjects were once again supplemented with the same dose for further 12 weeks (i.e., 25 to 36 weeks; phase II). The objective of the washout phase was to assess the impact of off-supplementation period on serum 25(OH)D concentration and thereby know the periodicity of supplementation required. The duration was based on considering 2-3 weeks half-life of 25(OH)D in literature [18]. Subjects were instructed to take VD capsules at any time of day with a cup of non-fortified milk. For subjects taking once monthly doses, capsules were given under supervision of the investigator. Subjects were advised to report any adverse reactions (pain abdomen, nausea, vomiting, dysuria, graveluria, hematuria) immediately either in person or telephonically to their treating physician-in-charge (SS and MAG) and if so, serum total Ca and urine calcium/creatinine (Ca/Cr) was checked immediately. Otherwise, all subjects had periodic monitoring of serum total Ca and urine Ca/Cr at 12, 24 and 36 weeks. During the course of trial, only three subjects (two in group C and one in group D) had asymptomatic hypercalciuria which was managed by stoppage of oral cholecalciferol. Since hypercalciuria settled in all three subjects, supplementation was restarted in one week. Treatment adherence was ensured by sending SMS and /or telephone calls to all the subjects every two weeks. Among daily-dose arms monthly pill count with return of empty bottles was enforced while in the group D (once monthly dose) VD was administered monthly under direct supervision. During washout phase, no capsules were provided to the subjects and were emphasized not to take any VD or Ca supplements.

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Clinical Assessment: All the enrolled subjects were clinically examined by single clinician (SS) recording general physical examination including anthropometry, blood pressure etc. Anthropometric measurements were performed using standard methodologies and instruments (SECA 13, Hamburg, Germany) as was for blood pressure (Omron HEM7120, Omron Corporation, Kyoto, Japan). Participants were advised to follow stable diet and exercise routine and were advised not to use sunscreens or any drug or additional health supplements likely to effect Ca or VD metabolism unless deemed necessary.

Laboratory evaluation: After an overnight (8-12 hours) fast, about 10 ml venous blood sample was drawn for measurement of serum total calcium (Ca), phosphorus (PO4), alkaline phosphatase (ALP), urea, creatinine (Cr), albumin, 25(OH)D, and intact parathyroid hormones (iPTH). The blood samples were collected in plain, ethylenediamine tetra acetic acid and fluoride vacutainers depending upon the assay and the aliquots were transported as soon as possible in cold boxes to the lab. The samples were aliquoted for immediate biochemical estimations, while as for hormonal analysis samples were stored at -80^{o}C until the assay. Serum total Ca, PO4, ALP, urea, Cr, albumin was measured using commercially available kits on an automated biochemistry analyzer (Response-910 Diasys Diagnostic systems, Holzheim, Germany). The serum 25(OH) D and iPTH were measured by electrochemiluminescence assay (COBAS e411, Roche Diagnostics Limited, USA) with respective ranges as 3-100 ng/ml and 10–65 pg/ml. The external quality control for serum 25(OH) D assay in our laboratory is done by participating in Randox International Quality Assessment Scheme (RIQAS), with intra- and inter-assay coefficients of variation (CV) for repeatability as 3·5 and 5% for serum 25(OH)D and 2·4 and 3·6% for serum iPTH respectively. Similarly, the CV for precision was 3.8-8.9% and 1.7 and 2.0 % respectively for serum 25(OH) D and iPTH. The accuracy of serum 25(OH)D assay in our laboratory is around 95%. Spot urine samples were also collected for the Ca/Cr ratio (both serum Ca and Cr measured in mg) measured using an automated analyzer (Beckman Coulter, Inc., CA, USA).

Follow up: All participants were subjected to measurements of repeat serum Ca, PO4, ALP, 25(OH)D, iPTH and urinary Ca/Cr at each follow-up visit i.e., 12 weeks, 24 weeks and 36 weeks. The rate constant defined as change in serum 25(OH)D concentration per 100 IU ingestion was calculated as follows: Serum 25(OH)D at 36 weeks minus serum 25(OH)D at baseline divided by total units of cholecalciferol ingested daily in IU multiplied by 100.

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Outcomes: The primary outcome measure was percentage of subjects achieving VD sufficiency i.e., serum 25(OH)D >20 ng/mL at the end of 36 weeks after supplementation with 400, 1000, 2000 IU VD daily and 60,000 IU cholecalciferol monthly. The secondary outcome was the change in concentrations of serum 25(OH)D**,** iPTH , Ca, PO4, ALP and any adverse events (hypercalcemia i.e. serum total Ca >10.5 mg/dl, hypercalciuria i.e. spot urine Ca/Cr ratio >0.2 mg/mg) at the end of 36 weeks.

Sample size calculation:

A sample size of 84 subjects, 22 in each group, is adequate to detect a clinically important difference assuming the small effect size (partial eta squared=0.03) in assessment of a primary outcome serum 25(OH)D concentration assuming a correlation among the repeated measures 0.5 and (non-sphericity correlation=1) using repeated measures ANNOVA, within-between interaction at 90% power and a 5% level of significance. Considering a high attrition rate during the study either due to withdrawal or noncompliance, we enrolled 153 subjects in the study (Group A: n=37, Group B: n=37, Group C: n=40, Group D: n=39).

Data analysis: All statistical analyses were performed using SPSS software, version 22 (SPSS Inc., Chicago, IL, USA). The normality of all the variables was tested using Kolmogorov–Smirnov test and all the variables in four groups were found to be normally distributed. Baseline variables among different groups were presented as mean and standard deviation (SD), if not stated otherwise. The missing data at different time points was handled using simulation based multiple imputations method using regression analysis. Variables were summarized with repeated measures ANOVA for comparisons within and between group effects on the key outcomes. The model include intervention group (4 treatment groups) x time (4 time points) as fixed factors for the outcome measures [serum 25(OH)D, serum iPTH, serum Ca, serum PO4, serum ALP, and urine Ca/Cr ratio)]. If there were significant interactions, the post-hoc analysis was done using Tukey's HSD (equal variances) or Games-Howell (unequal variances). The homogeneity of variance (Sphericity) was checked by the Mauchly's test of sphericity. The Greenhouse-Geisser correction was done in case sphericity assumption was violated. We used a generalized linear model (binomial distribution) with the dependent variable as VD sufficiency (Yes/No) to find the difference in VD sufficiency status in different doses and different time points. Quoted P values are not adjusted for multiple comparisons. To preserve the original randomization and to avoid the bias due to exclusion of patients, the data was analyzed by intention to treat (ITT) analysis.

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All the tests were conducted two-sided, and a *p* value of <0.05 was considered statistically significant.

Results

Of total 230 subjects screened, nine refused to participate and 221 were clinically evaluated, out of which 68 were excluded either because their serum 25(OH)D concentrations were >20 ng/mL (n=53) or had systemic illnesses (n=15). The remaining 153 subjects were enrolled and randomized into four groups (Group A: n=37; Group B: n=37; Group C: n=40; Group D: n=39) to receive oral cholecalciferol doses of 600 IU/day, 1000 IU/day, 2000 IU/day or 60,000 IU/month respectively (Figure 1). No significant difference was reported in age, clinical, anthropometric and biochemical characteristics at baseline (Table 1). Overall, in the screened population (n=221), 69.2% of the subjects were VD deficient. The average daily sun exposure of screened subjects was 40±12 minutes with average body surface area exposed to sun was 8.75%. During the course of trial, twelve subjects were lost in follow up and ten subjects were withdrawn from the final analysis due to non-compliance (Figure 1).

A significant time-group interaction was found in serum 25(OH)D concentration. Post hoc analysis revealed that serum 25(OH)D concentration increased significantly from baseline to 12 weeks (phase I of supplementation) in all the groups. At 24 weeks (end of washout phase), no change in serum 25(OH)D concentrations was observed compared to 12 weeks. A further increase in serum 25(OH)D concentration was observed at 36 weeks (end of phase II of supplementation) in all the groups (Table 2). Group C had a significantly higher increase in serum 25(OH)D concentration at 12 and 36 weeks compared to other groups (Table 2). The overall mean rate constant of study subjects was 1.7±0.7 ng/ml/100 IU. At the end of study, 65%, 78%, 95%, and 90% of the subjects in groups A, B, C and D respectively were VD sufficient (Table 3 and supplementary Table 1). However, no difference was observed in group Cand D in proportion of subjects achieving VD sufficiency.

Serum iPTH decreased significantly from baseline to 12 weeks, with no change during washout phase and a further significant decrease from 24 to 36 weeks, with no time-dose interactions. Similarly, no significant time-dose interactions were observed in serum Ca, PO4 and ALP or Urine Ca/Cr ratio (Table 2). No major adverse events were recorded during this trial except asymptomatic hypercalciuria in three subjects (two subjects in group C and one in group D) at 12 weeks which subsided after stopping oral cholecalciferol for one week.

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Discussion

The pursuit to arrive at an optimal daily dose of VD supplementation to prevent VD deficiency continues because of discrepant recommendations for adults by NAM, ICMR (600IU /day) and ES (1500-2000 IU /day)[5,7,8] generating significant debate [12,19]. Among plethora of studies in literature only few are comparable due to varying duration and doses of VD supplementation, ages and gender of subjects as well as cut-off levels taken to define sufficiency [15,20–25]. There are no studies reporting impact of daily oral cholecalciferol supplementation on serum 25(OH)D concentrations among Indian adults. In view of the above, we undertook this prospective randomized trial comparing three different daily oral doses versus monthly oral bolus of cholecalciferol among VD deficient adults.

High prevalence (68.9%) of VD deficiency in the present study with an average daily sun exposure of 40±12 minutes over 8.75% (0-14) body surface area, reiterates the reported widespread nature of VD deficiency among Indians [2–4]. On daily oral supplementation with 600 and 1000 IU of cholecalciferol, 65% and 78% subjects achieved VD sufficiency, leaving a significant percent of population VD deficient at the end of 36 weeks. These observations are in contrast to those reported by Gallagher et al [22] and Lehman et al [26], who demonstrated that supplementing with 800 IU per day oral cholecalciferol achieved VD sufficiency in approximately 94% subjects. Both these studies were conducted in non-Asian subjects and their baseline serum 25(OH)D was higher than the current study. In addition the duration of treatment was 12 months in Gallagher etal [22] as against 6 months in current study. Likewise, Cashman et al [27] in a meta-regression analysis of 44 non-Asian studies also worked out a supplementation dose of 930 IU/day for achieving serum levels of 25(OH)D >50 nmol/L or 20 ng/ml. Several studies suggested suboptimal efficacy of doses ranging from (400 to1000 IU daily) among non-Indian adult subjects [20,21,24,25,28] consistent with the results of the present study. In one of our studies, Indian children could achieve VD sufficiency in 71.5 % and 81.8% with daily supplementation of 600 and 1000 IU for 6 months [13]. In most of these studies, baseline serum 25(OH)D was lower as well as duration of treatment was less than 6 months.

The major observation of the present study was the adequacy of 2000 IU in achieving VD sufficiency among 95% subjects, consistent with other studies wherein VD was supplemented for three to 24 months among non-Asians adult subjects [24,25,29,30]. Few authors, however, did report suboptimal efficacy at equivalent or even higher than 2000 IU/day prescribed in this

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study, especially in pregnancy, lactation and patients with diabetes mellitus or fractures [21,31–33]. The dose dependent increase of 35.74 ng/ml in serum 25(OH)D concentration with 2000 daily IU in the current study was similar to what was observed in earlier study [32] but higher than that observed by Chandler et al (19.2 ng/ml) [24] and Shieh et al (13.8 ng/dl) [34] likely because of higher BMI, higher baseline serum 25(OH)D and shorter duration of treatment in these studies.

The mean rate constant of 1.7±0.7 ng/ml/100 IU in our study was higher than that reported by Holick M F et al [8] but lower (2 ng/ml/100 IU) than that reported by McKenna et al [35] in severely VD deficient elderly subjects. This supports the fact that baseline 25(OH)D concentration determine the rate constant inversely. This lower rate constant may also be explained by altered VD metabolism in Asian/ Indian population [36] with documented increased 24 hydroxylase activity which may increase metabolism of 25(OH)D and decrease its concentrations.

VD sufficiency achieved in 90% subjects with monthly dose of 60,000 IU is more or less similar (95%) to that achieved with oral dose of 2000 IU/day, though with a higher increase in serum 25(OH)D concentration in group C. This finding is supported by reports from Niet et al in adults [29] and Marwaha et al [37] among others [31,38–40] who documented 100% and 90% subjects attaining serum 25(OH)D concentration >20 ng/ml respectively with 60,000 IU and 50,000 IU oral monthly doses. These results assume significance in view of the fact that once monthly cholecalciferol supplementation may be convenient for people and ensure better compliance.

The interesting point of this study was observing washout period from week 13 to 24 to observe time dependency of VD supplementation and impact of baseline concentration on its kinetics. The initial supplementation for 12 weeks increased serum 25(OH)D concentration significantly in all groups rendering about 22%, 60%, 95% and 64 % VD sufficient in groups A, B C and D respectively. However, stopping supplementation for 3 months did not change serum 25(OH)D concentration, contrary to our expectations. This may be probably due to half-life of VD and may suggest the interval of bolus supplementation. Further supplementation in phase II (week 25-36) lead to VD sufficiency in 65%, 78%, 95% and 90% in groups A, B, C and D respectively at 36 weeks. Carefully observing dose response VD trials, reveal that doses around 1000 IU may achieve sufficiency if given for longer periods, while as higher doses >1000 IU renders subjects VD sufficient in around 12 weeks.

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[27,41,42].Thus, we can conclude that not only dose but duration of treatment is also important while considering VD supplementation.

Though no time-dose interaction was observed in serum iPTH concentration during the trial period, but serum iPTH concentration decreased significantly at 12 weeks in all groups with no change at 24 weeks but a further decrease at 36 weeks. Some studies [23,43] have observed dose and time-dependent decrease in serum PTH after VD supplementation, but the data suggests that the PTH decreasing tendency of VD pleatues around 1000 IU daily [43]. Except that three subjects (two in group C and one in group D) developed transient hypercalciuria which settled after one week, no major side effects were reported. To the best of our knowledge, this is the first randomized study simultaneously evaluating efficacy of three oral daily doses and once monthly bolus dose of cholecalciferol among VD deficient Indian adults. The study shows that 2000 IU daily or 60,000 IU monthly doses of oral cholecalciferol achieve VD sufficiency in higher percentage of subjects (95% vs 90%) than 600 and 1000 IU daily with no major adverse events. Though 2000 IU daily cholecalciferol supplementation had a higher increase in serum 25(OH)D concentration, but once monthly dose may have better compliance. The results may not, however, be generalizable to population with low prevalence of VD deficiency. However certain limitations of this study are: a) inability to evaluate bone formation and resorption markers, b) spot Ca/Cr ratio instead of twenty-four-hour urine Ca excretion was evaluated to gauge hypercalciuria, c) lack of placebo group as it would provide absolute effect of supplementation on serum 25(OH)D concentration. However, it would have been unethical not to supplement subjects who were VD deficient, d) having one arm of study as open label (once monthly 60,000 IU), e) confounding effect due to seasonal variation in sun exposure but the recruitment window in our study was relatively narrow and we did not expect any gross differences in the results, f) lack of standardised assessment of adverse events whereby some minor side-effects were not captured, and g) Taking cholecalciferol capsules with cup of milk though not fortified with VD, may have a confounding effect on 25(OH)D concentration especially in Group D and during washout phase .

We conclude that while reiterating the high prevalence of VD deficiency among Indian adults, the study demonstrates that 2000 IU oral cholecalciferol daily for 6 months is safe and efficacious among VD deficient Indian adults. Administering this as a single monthly dose of 60000 IU orally is a plausible option.

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Author contribution: MAG, RKM, IAW, RAM designed research; SS, TS, AR, IAW conducted study; MAG, MSB, TS, ARW, SS, MMA analyzed data; MAG: MSB; SS; TS; RAM wrote manuscript; and MAG was primarily responsible for final content. All authors have read and approved the final manuscript.

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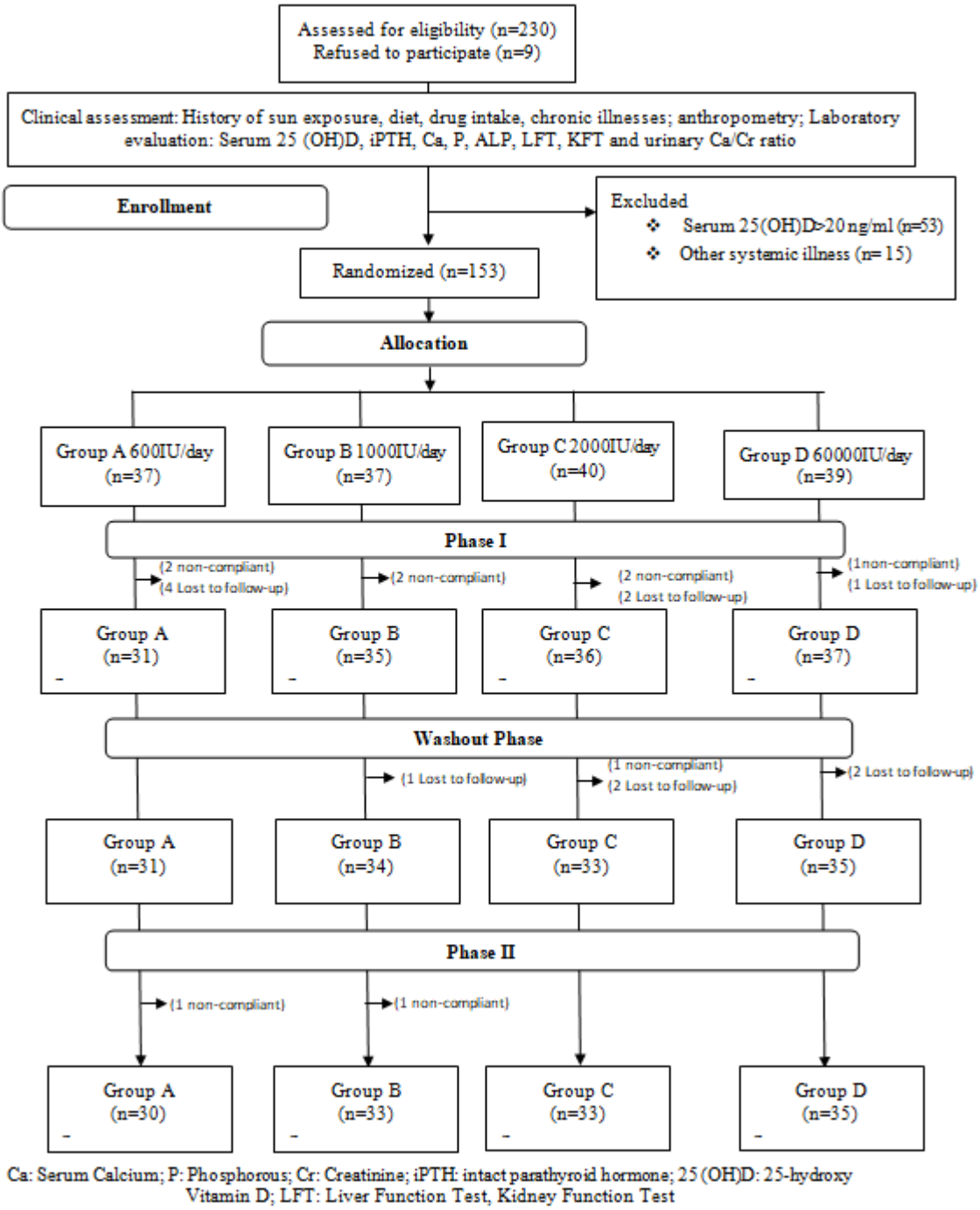

Ca: Serum Calcium; P: Phosphorous; Cr: Creatinine; iPTH: intact parathyroid hormone; 25 (OH)D: 25-hydroxy Vitamin D; LFT: Liver Function Test, Kidney Function Test

Figure 1: Enrolment and randomization

Ca: Serum Calcium; P: Phosphorous; Cr: Creatinine; iPTH: intact parathyroid hormone; 25 (OH)D: 25-hydroxy Vitamin D; LFT: Liver Function Test, Kidney Function Test

Table 1: Demographic and baseline characteristics of study participants

Parameter	**Group A** (600IU/day) (N=37)	**Group B** (1000IU/day) (N=37)	**Group C** (2,000IU/day) (N=40)	**Group D** (60,000IU/month) (N=39)
Age (years)	28.95±11.08	30.32±11.27	29.13±10.01	34.44±13.68
Male/ Female	12/25	15/22	15/25	17/22
BMI (Kg/m^2)	21.75±3.34	22.49±2.96	22.97±3.03	23.2±3.18
Blood urea (mg/dl)	24.23±5.3	23.62±5.55	23.68±5.75	24.06±5.73
Serum creatinine (mg/dl)	0.76±0.18	0.8±0.18	0.79±0.19	0.84±0.23
Serum alkaline phosphatase (IU/L)	91.07±24.91	93.68±28.99	91.5±20.31	96.61±24.64
Serum albumin (g/dl)	4.81±0.4	4.82±0.49	4.84±0.37	4.67±0.51
Serum calcium-total (mg/dl)	9.62±0.56	9.72±0.47	9.75±0.54	9.76±0.48
Serum phosphorus (mg/dl)	3.25±0.42	3.27±0.4	3.22±0.36	3.26±0.36
Serum 25 (OH)D (ng/ml)	11.4±3.8	11.1±4.4	12.8±3.9	11.4±4.3
Plasma iPTH (pg/ml)	54.21±21.82	53.67±20.36	49.72±23.15	49.82±20.95
Urine calcium/creatinine (mg/mg)	0.14±0.04	0.13±0.04	0.14±0.05	0.11±0.06

BMI: Body mass index; iPTH: intact parathyroid hormone; 25 (OH)D: 25 hydroxy vitamin D; One-way ANOVA test was performed to compare groups

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Table 2: Trajectory of bone mineral parameters with different oral cholecalciferol dosing regimen and schedules

Parameters	0 Weeks (Baseline)	12 Weeks (Phase I)	24 Weeks (Washout)	36 Weeks (Phase II)	P Value*
Serum (OH)D Levels (ng/ml)					
Group A (600 IU/day)	11.4±3.8	18.6±5.4[a]	17.5±5.5	26.0±9.1[c]	<0.01
Group B (1000 IU/day)	11.1±4.4	20.9±6.2[a]	19.7±6.5	30.9±9.7[c]	
Group C (2000 IU/day)	12.8±3.9	31.9±7.8[avx]	30.0±7.5[vx]	47.8±10.9[cvx]	
Group D (60,000 IU/month)	11.4±4.3	25.7±9.99[awz]	24.1±8.9[wz]	38.4±12.8[cwyz]	
Serum iPTH (pg/ml)					
Group A (600 IU/day)	54.21±21.82	45.58±18.62[a]	45.21±16.79	35.94±20.88[c]	0.404
Group B (1000 IU/day)	53.67±20.36	42.84±19.97[a]	43.72±19.02	32.98±18.07[c]	
Group C (2000 IU/day)	49.72±23.15	34.9±13.33[a]	35.23±13.11	28.97±14[c]	
Group D (60,000 IU/month)	49.82±20.95	39.99±18[a]	39.17±17.1	32.84±15.98[c]	
Serum Calcium (mg/dl)					
Group A (600 IU/day)	9.62±0.56	9.7±0.46	9.67±0.42	9.72±0.42	0.496
Group B (1000 IU/day)	9.72±0.47	9.6±0.46	9.63±0.42	9.69±0.39	
Group C (2000 IU/day)	9.75±0.54	9.72±0.55	9.8±0.42	9.84±0.42	
Group D (60,000 IU/month)	9.76±0.48	9.77±0.41	9.78±0.38	9.8±0.34	

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Serum phosphorus (mg/dl)					
Group A (600 IU/day)	3.25±0.42	3.43±0.46	3.48±0.44	3.55±0.37	0.990
Group B (1000 IU/day)	3.27±0.4	3.43±0.5	3.46±0.48	3.59±0.55	
Group C (2000 IU/day)	3.22±0.36	3.39±0.37	3.4±0.4	3.5±0.44	
Group D (60,000 IU/month)	3.26±0.36	3.44±0.4	3.51±0.41	3.59±0.4	
Serum ALP (IU/L)					
Group A (600 IU/day)	91.07±24.91	85.66±26.28[a]	85.86±24.31	81.92±23.5[c]	0.605
Group B (1000 IU/day)	93.68±28.99	85.45±25.42[a]	87.05±23.75	79.99±25.35[c]	
Group C (2000 IU/day)	91.5±20.31	85.22±22[a]	86.66±21.08	78.41±16.9[c]	
Group D (60,000 IU/month)	96.61±24.64	87.22±18.67[a]	86.87±18.41	82.28±18.54[c]	
Urine Ca/Cr Ratio (mg/g)					
Group A (600 IU/day)	0.14±0.04	0.12±0.07	0.11±0.06	0.12±0.07	0.397
Group B (1000 IU/day)	0.13±0.04	0.13±0.05	0.11±0.06	0.14±0.11	
Group C (2000 IU/day)	0.14±0.05	0.14±0.04	0.15±0.06	0.15±0.04	
Group D (60,000 IU/month)	0.11±0.06	0.12±0.04	0.11±0.04	0.12±0.05	

a: $p<0.05$ Baseline vs. Phase I; c: $p<0.05$ Wash out vs. Phase II; u: Group B vs. A; v: Group C vs. A; w: Group D vs. A; x: Group B vs. C; y: Group B vs. D; z: Group C vs. D; Analysis by ITT; *: P value for time by group interactions; P<0.05 statistically significant; Analysed using repeated measures ANOVA; $p<0.05$ is considered as significant; iPTH : intact parathyroid hormone; 25 (OH)D: 25 hydroxy vitamin D; ALP: alkaline phosphatase; Ca/Cr; calcium/creatinine

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Table 3: Proportion of subjects with sufficient serum 25 (OH)D at different phases of supplementation

	Phase-I (12 weeks) n(%)	**Washout (24 weeks) n(%)**	**Phase-II (36 weeks) n(%)**	**P Value***
Group A (600 IU/day) (N=37)	8 (21.6)[a]	7 (18.9)	24 (64.9)[c]	
Group B (1000 IU/day) (N=37)	22 (59.5)[au]	19 (51.4)[u]	29 (78.4)[c]	<0.05
Group C (2000 IU/day) (N=40)	38 (95.0)[avx]	36 (90.0)[vx]	38 (95.0)[vx]	
Group D (60,000 IU/month) (N=39)	25 (64.1)[awz]	25 (64.1)[wz]	35 (89.7)[cwy]	

a: $p<0.05$ Baseline vs. Phase I; c: $p<0.05$ Wash out vs. Phase II; u: $p<0.05$ Group A vs. B; v: $p<0.05$ Group A vs. C; w: $p<0.05$ Group A vs. D; x: $p<0.05$ Group B vs. C; y: $p<0.05$ Group B vs. D; z: $p<0.05$ Group C vs. D VD sufficient >20ng/ml; Wald test ; $p<0.05$ is considered as significant. *: P value for group-by-time interactions

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