British Journal of Nutrition (2023), 129, 1473–1480 doi:10.1017/S0007114522001477

Daily *v.* weekly oral vitamin D_3 therapy for nutritional rickets in Indian children: a randomised controlled open-label trial

Aashima Dabas[1]*, Vineet Dabas[2], Pradeep Kumar Dabla[3], Raman Kumar Marwaha[4], Sangeeta Yadav[1], Madhavi Bharadwaj[1], Vernika Tyagi[1] and Shipra Agrawal[1]

[1]*Department of Pediatrics, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi, India*
[2]*Department of Orthopedics, Lady Hardinge Medical College and Associated Hospitals, New Delhi, India*
[3]*Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi, India*
[4]*Consultant Endocrinologist and President, Society for Endocrine Health of Elderly, Adolescents and Children, New Delhi, India*

(Submitted 1 September 2021 – Final revision received 10 April 2022 – Accepted 3 May 2022 – First published online 13 May 2022)

Abstract

The aim of the study was to compare the efficacy of daily *v.* weekly oral vitamin D_3 therapy in radiological healing of nutritional rickets. Children 6 months to 12 years (*n* 132) diagnosed with nutritional rickets were randomised into three groups (*n* 44): group A – 2000 IU daily vitamin D_3 for 12 weeks, B – 60 000 IU weekly for 3 weeks, C – 60 000 IU weekly for 6 weeks. Serum calcium, phosphorus, 25-hydroxyvitamin D (25(OH)D), parathyroid hormone and X-ray score were estimated at baseline and 12 weeks (endline). The proportion of children who achieved complete radiological healing at endline was compared between three groups by χ^2 and delta change in laboratory parameters by ANOVA (parametric data) or Kruskal Wallis test (non-parametric data), respectively. Baseline 25(OH)D ≤ 20 ng/ml was seen in 119 (90·2 %), hyperparathyroidism in 90 (68·8 %) and hypocalcaemia in 96 (72·7 %). A total of 120/132 children completed the study. Complete radiological healing seen in 30 (75 %) in group A, 23 (60·5 %) in group B and 26 (61·9 %) in group C; $P = 0{\cdot}15$, with comparable endline X-ray scores; $P = 0{\cdot}31$. The median (interquartile range (IQR)) delta X-ray score (baseline–endline) was 7 (4,9), 5 (2·25, 6) and 6 (4,7) in groups A, B and C, respectively; $P = 0{\cdot}019$. Median (IQR) 25(OH)D endline levels in groups A, B and C were 50·0 (26·5, 66·5), 42·1 (28·4, 54·4) and 53·5 (33·7, 71·2) ng/ml, respectively; $P = 0{\cdot}045$. Radiological scores were comparable at endline among daily and weekly vitamin D groups with greater change from baseline in daily supplemented group.

Key words: **Cholecalciferol: Stoss therapy: Vitamin D deficiency: Hypocalcaemia: 25-hydroxyvitamin D**

Nutritional rickets is a common bone disorder in childhood characterised by skeletal deformity, abdominal distension, delayed motor milestones and seizures. The treatment of rickets consists of administration of both Ca and vitamin D. Traditionally, the Stoss regimen was used to treat rickets which involved administration of mega doses (600 000 IU) of vitamin D with Ca[1]. This regimen was cost-effective and ensured compliance; however, the risk of nephrocalcinosis and hypervitaminosis D with this regimen remained a major concern. The efficacy of lower doses of vitamin D[2–7], and staggered daily[8] or weekly[7,9] regimens were evaluated and found effective for treatment of nutritional rickets. Oral route was increasingly favoured over intramuscular route of vitamin D administration with similar efficacy and minimal risk of toxicity[10,11].

The guidelines on treatment of nutritional rickets recommend age-based daily or weekly/bi-weekly dose of oral vitamin D for treatment of nutritional rickets in children[10–12]. Daily regimen has lower chances of vitamin D toxicity than bolus regimen with comparative doses; however, compliance and cost remain major concerns for its routine use[10–12]. There are limited trials in children which have compared the efficacy of daily or weekly low-dose vitamin D regimen in rickets[7]. The best single dose which can achieve radiological healing and rise in serum 25-hydroxyvitamin D (25(OH)D) levels still needs to be ascertained in Indian children who have higher melanin content and poor Ca and vitamin D intake[13].

We, therefore, conducted this study to compare the efficacy of 2000 IU vitamin D_3 administered daily for 12 weeks, with 60 000 IU weekly (three or six doses for cumulative dose of 180 000 or 360 000 IU, respectively) oral vitamin D_3 for treatment (radiological healing) of nutritional rickets in children aged 6 months to 12 years. The improvement in serum 25(OH)D

Abbreviations: ALP, alkaline phosphatase; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

* **Corresponding author:** Aashima Dabas, email dr.aashimagupta@gmail.com

levels and other biochemical parameters (parathyroid hormone (PTH), Calcium, alkaline phosphatase (ALP)) were also measured.

Methods

This study was conducted from June 2018 to February 2020 in the Department of Pediatrics and Orthopedics of a large public hospital located in northern India. An approval from the Institutional Ethics Committee of the Institute was obtained before commencing the study. Written informed consent was obtained from the parents for participation and assent for examination was obtained from children older than 7 years. The trial was registered prospectively at www.ctri.nic.in (CTRI/2018/04/013300).

All children aged 6 months to 12 years who presented to the hospital with a diagnosis of nutritional rickets defined by clinical, biochemical and radiological parameters, either singularly or in combination[10–12] and residing within 50 km of hospital who are willing for follow-up visits were included. Any patient with confirmed or suspected diagnosis of malabsorption or chronic kidney or hepatic disease as per history and clinical examination, patients with severe systemic illness compromising oral intake (tachycardia, tachypnoea, shock, weak peripheral pulses, increased capillary refill time) or patients who had taken Ca supplements or vitamin D preparation in last 6 months were excluded.

Randomisation and allocation concealment

This was a parallel three group randomised controlled open-label trial. Block randomisation was done in three groups A, B and C using randomly permuted blocks of size 3 and 6 as per a randomisation sequence generated at www.randomization.com by a person not related to the study. This randomisation sequence was transcribed to sequentially numbered opaque and sealed envelopes by the person not directly related to the study. At the time of enrolment, the envelopes pertaining to the sequence number of the patient were opened by one of the study investigators and group allocation was done.

Intervention

Patients were randomised into three groups A, B and C. Vitamin D preparations available as a nanoemulsified formulation (ready to administer) of a single batch were used in this study. Group A received oral liquid preparation containing 2000 IU (2·5 ml) vitamin D_3 daily available as liquid (DePURA kids nano drops 15 ml, 1 ml/800 IU, Sanofi India Ltd) in monthly rations for 12 weeks[11,12]. The daily dose was kept as 2000 IU irrespective of patients' age for feasibility of drug administration. Group B received oral dose of 60 000 IU for three consecutive weeks (cumulative dose – 180 000 IU) after enrolment to evaluate the effect of a dose approximately equivalent to the daily oral dose of 2000 IU for 12 weeks. The dose in group B also evaluated the efficacy of supplementing a lower dose of vitamin D in this study group. Group C received an oral dose of 60 000 IU for six consecutive weeks (cumulative dose – 360 000 IU) after enrolment as advocated for treatment of nutritional rickets[11]. Patients in group B and C were given oral formulation containing 60 000 IU vitamin D_3 (DePURA oral solution, 5 ml/60 000 IU, Sanofi India Ltd) to be administered once a week (on a fixed day every week) for 3 or 6 consecutive weeks, respectively, to complete the required vitamin D dose. The oral vitamin D_3 solution was administered directly to the child without dissolving it in any liquid. Three doses of these were dispensed at one time, that is, patients in group C were required to collect their medication twice from the hospital, while those in group A collected their medicines every three weekly. The first dose of the drug in each of the three groups was administered supervised by the study team to observe for any vomiting, spillage. The mother/caregiver was instructed to administer the remaining doses at home. Patients were instructed to avoid any energetic intake for at least 30 min after vitamin D intake for optimal absorption. In case the patient vomited within half hour of taking treatment, the dose was repeated and fresh additional dose was collected in the same week from the hospital. This information was solicited from the caregiver at the consecutive hospital visit by the study team.

In addition to vitamin D_3, all patients of nutritional rickets received supplemental Ca (dispensed as plain Ca carbonate syrup for younger than 5 years old and tablet for older children) at dose of 500 mg/d or 50 mg/kg per d to maximum of 500 mg for total 12 weeks. Children younger than 5 years also received a maintenance dose of vitamin D 400 IU/d (DePURA kids nano drops 15 ml, 1 ml/800 IU, Sanofi India Ltd) and calcium 500 mg/d for 6–12 weeks after completion of therapeutic regimen (not part of study outcomes)[11]. Besides, the study subjects also received dietary counselling for optimum infant and young child feeding practices.

The clinical history, developmental history (for age-appropriate milestones), feeding history, daily intake of milk and dairy products by 24-h recall method and clinical examination were recorded at the time of enrolment. The date of birth and birth weight were recorded from the hospital records at birth. The Ca intake was estimated for animal/formula milk, other dairy products and eggs from 24-h recall as per available national food composition tables[14]. Weight was measured with a digital weighing machine in minimal clothing (without footwear in older children) and corrected to the nearest 0·5 kg. Length was measured till 24 months of age using an infantometer placed on a hard surface after placing the child in standard position. Height was measured in children older than 24 months with wall mounted Holtain's stadiometer (Holtain Inc., Crymych, Pembs.) with the measurement corrected to the nearest 3 mm, and interpreted as per national standards[15,16].

The laboratory quality assurance was followed as per NABL (National Accreditation Board for Testing and Calibration Laboratories) ISO 15189:2012 guidelines. Total 3 ml of blood was collected at baseline for estimation of serum calcium (total), phosphorus, ALP, serum 25(OH)D in a plain vacutainer (without any additives for collection of serum) and for intact PTH in a EDTA-coated vacutainer. The serum aliquot was centrifuged and serum was separated. A part of this was processed on same day for estimation of serum calcium, phosphorus and ALP in Department of Biochemistry by Roche Cobas c501 autoanalyser at the study site. Roche Cobas c501 autoanalyser module (routine testing) is a mid-volume analyser comprising a photometric unit for a broad range of clinical chemistry assays and an ISE unit

for ion-selective electrode. Normal range for serum calcium was 9–11 mg/dl (6–24 months) and 8·8–10·8 mg/dl in > 24 months of age. Normal range for serum phosphorus was 3·8–6·5 mg/dl (6–24 months), 3·2–5·8 mg/dl in older children. Normal range for serum ALP was 150–420 IU/l in infants and 100–320 IU/l in older children[17].

The remaining serum was separated and stored at −20°C until batch analysis for the estimation of 25(OH)D. Plasma sample was stored for analysis of PTH. Serum 25(OH)D and plasma PTH levels were measured with Roche immunoassay auto-analyser Cobas e411 using the electrochemiluminescence technique (Elecsys-2010, Roche Diagnostics). The minimum detection limit for PTH was 1·20 pg/ml (reporting reference interval 15–65 pg/ml) and for 25(OH)D was 3 ng/ml with the analytical measurement range of 3–100 ng/ml. Serum 25(OH)D levels were defined as sufficient if > 20 ng/ml, insufficient 12–20 ng/ml and deficient if < 12 ng/ml[10–12].Vitamin D intoxication was defined as serum 25(OH)D > 100 ng/ml with hypercalcaemia and/or hypercalciuria[10–12]. Values above 100 ng/ml were estimated by manual dilution as per the kit instructions. Suppressed PTH levels were defined as PTH levels below 10 pg/ml and secondary hyperparathyroidism was defined as PTH levels > 65 pg/ml.

A baseline spot urinary sample for urinary calcium: creatinine ratio (UCaCr) was taken in every fifth child for logistic issues. A cut-off value of UCaCr > 0·6 in infants, > 0·4 in children 12–24 months and > 0·2 in children older than 24 months was defined as hypercalciuria[18]. Baseline radiographs of both wrists and knees were taken to assess the severity of rickets through a standard radiological ten-point scoring system devised to assess the degree of metaphyseal fraying and cupping and the proportion of the growth plate affected. Score of 10 indicated severe rickets[19]. The radiological scoring was undertaken by a study personnel blinded to randomisation.

Follow-up

The follow-up visits were done at 3 weeks ± 6 d, 6 weeks ± 6 d and 12 weeks ± 1 week. Patient's compliance in the three groups (A, B and C) was recorded by parental recall and monitoring empty vials. Children who missed drug dosage for less than 7 d period in group A were asked to resume medication from next consecutive day (to complete a total of 12 weeks dosage) with the presumption that this would not affect skeletal healing. Treatment in group A was discontinued if a child defaulted for seven or more days consecutively presuming that this would optimise skeletal healing. Likewise, missed drug in group B/C was instructed to be taken on the next consecutive day within next 6 d. Treatment in group B/C was discontinued if a child missed weekly dosage for 1 week or more. At each follow-up visit, patients were enquired about any possible symptoms of hypercalcaemia such as abdominal pain, vomiting and headache. A radiograph of knee and wrist was repeated at 12 weeks to document improvement in radiological scores by a blinded assessor. A radiograph of the knee and wrist was also taken at 6 weeks in children who consented for it, to assess for radiological line of healing in rickets which appeared by 6 weeks[20]. The radiological improvement at 6 weeks was defined by appearance of a radio-opaque line at metaphyseal ends irrespective of delta change in the radiological score. Complete radiological healing was defined as a radiological score less than or equal to 1·5 at 12 weeks[19].

Composite healing was defined by the authors as improvement in clinical symptoms, increase in serum 25(OH)D levels to > 20 ng/ml and improvement in the radiological score 50 % or more from the baseline (all three criteria). A 2-ml blood sample repeated for measurement of serum calcium, phosphorus, ALP, 25(OH)D and PTH was taken at 12 weeks. The urinary spot sample was repeated for UCaCr in the limited subset. Patients who did not achieve complete radiological healing by 12 weeks were continued on the similar maintenance dose of vitamin D 400 IU/d with Ca 500 mg/d without any additional use of higher doses of vitamin D, to avoid the risk of hypervitaminosis D. It was presumed that this group of children could be those with more severe rickets and would take longer to achieve complete radiological healing. These children were followed up every 3–4 weekly (outcomes not as part of this study). Any child with suspected non-nutritional rickets[12] was excluded.

Outcomes

The proportion of children who achieved complete radiological healing (defined as radiological score < 1·5) and composite healing at 12 weeks across three regimens were compared. Delta X-ray score was calculated as score at baseline minus score at endline (12 weeks) and compared among three groups. The increase in serum 25(OH)D, decline in PTH level and ALP level at 12 weeks and proportion of children with vitamin D sufficiency defined as serum 25(OH)D > 20 ng/ml at 12 weeks were also compared among three groups.

Sample size

Sample size was calculated using the formula for sample size for experimental design for proportions. In a study by Chatterjee *et al.*[20] the proportion of children who achieved complete radiological healing at 12 weeks was 47 %. Assuming a difference of 20 % in proportion across intervention arms, the sample size with 80 % power and α error 5 % is forty subjects per arm. The total number of subjects required after accounting for 10 % lost to follow-up was forty-four subjects per arm.

Statistical tests and analysis

Data were entered in Microsoft excel and analysed using SPSS version 25. Descriptive representation of continuous data was done using mean, SD, median (interquartile range) and proportions. Student *t* test and Mann Whitney test were used to compare laboratory values (parametric and non-parametric, respectively) between those with and without vitamin D deficiency. Radiological scores and secondary outcome variables were compared across three groups using univariate ANOVA test for parametric data or Kruskal Wallis test for non-parametric data (with Dunn-Bonferroni correction for post hoc analysis). Friedman's two way analysis by rank test was used to compute paired analysis on non-parametric data across three groups. The comparison of paired data on laboratory values before and after within each

group was done using paired *t* test for parametric variables and Wilcoxon matched-pair signed rank test for non-parametric variables. Chi-square test was used to compare proportions between three groups. Analysis was done as per-protocol analysis for all outcomes except radiological score as 12 weeks (primary outcome) which was compared using intention to treat analysis. Pearson or Spearman's correlation coefficient was used to measure correlation between continuous variables like radiological scores and laboratory parameters. *P* value < 0·05 was considered as significant.

Results

A total of 132 out of 163 children were screened with median (interquartile range) age of 12 (8, 24) months; 120 of them completed the study (Fig. 1). Among them, seventy-four were infants (6–12 months of age), forty-nine children were between 13 and 60 months and 9 above 60 months of age. The most common clinical features included skeletal abnormalities in 108 (81·8 %), developmental delay in 39 (29·5 %) and hypocalcaemic seizures in 32 (24·2 %). Twelve children had concurrent pneumonia and two had concurrent diarrhoea. The mean breastfeeding duration was 6·14 (SD 4·0) months and only 47 (35·6 %) children were exclusively breastfed for 6 months. Dietary Ca intake was < 500 mg/d in 88 (66·6 %) children with an overall mean of 333·12 (SD 202·86) mg/d; milk, dairy products and poultry eggs were the only sources of Ca in the children.

In all, 119 (90·2 %) subjects had vitamin D insufficiency (serum 25(OH)D ≤ 20 ng/ml) and 91 (68·9 %) had vitamin D deficiency (< 12 ng/ml) at baseline. Overall, vitamin D deficiency among groups A, B and C was observed in 34 (77·3 %), 27 (61·4 %) and 30 (68·2 %) subjects, respectively. The baseline characteristics of the study groups are shown in Table 1. Hypocalcaemia was observed in 96 (72·7 %), hypophosphatemia in 56 (42·2 %) and elevated ALP in 106 (80·3 %) children. The proportion of children with secondary hyperparathyroidism was 31 (70·5 %), 31 (70·5 %) and 28 (63·6 %) in groups A, B and C, respectively; $P = 0·73$. Baseline X-ray score had insignificant negative correlation with serum 25(OH)D ($r = –0·12$, $P = 0·19$) and serum calcium ($r = –0·13$, $P = 0·13$). A significant correlation was observed between serum 25(OH)D and serum calcium ($r = 0·296$, $P = 0·001$), serum ALP ($r = –0·172$, $P = 0·048$) and PTH ($r = –0·37$, $P < 0·001$).

The X-ray scores on follow-up are shown in Table 2. Forty (90·9 %), 38 (86·4 %) and 42 (95·5 %) children completed the study in group A, B and C, respectively, with similar radiological scores at 12 weeks (Fig. 2), but significantly different median (interquartile range) delta change in X-ray scores (baseline minus post-intervention). The biochemical variables after intervention are shown in Table 3. None of the patients who dropped out in either group reported any discomfort with the administered treatment but declined to pursue the intervention and follow-up at study site.

Complete radiological healing was detected in 79/120 (65·8 %) children and composite healing in 115/120 (95·8 %) children after 12 weeks. Children without radiological healing ($n = 41$) when compared with those with complete radiological healing ($n = 79$) were found to be younger with mean age (16·32 (SD 14·84) and 24·3 (SD 31·54) months; $P = 0·062$), with comparable mean dairy Ca intake (347·07 (SD 183·71) and 328·33 (SD 220·82) mg/d; $P = 0·645$), lower serum calcium (7·51 (SD 1·94) and 8·12 (SD 1·56) mg/d; $P = 0·085$), lower mean serum phosphorus (3·72 (SD 1·51) and 4·21 (SD 1·47) mg/dl; $P = 0·095$) and serum 25(OH)D at baseline (8·57 (SD 5·29) and 11·12 (SD 10·66) ng/ml; $P = 0·083$) with higher ALP (935·46 (SD 562·32) and 777·92 (SD 599·70) IU/ml; $P = 0·166$), PTH (216·76 (SD 150·98) and 143·85 (SD 133·24) pg/ml; $P = 0·008$) and baseline X-ray score (9·22 (SD 1·54) and 7·29 (SD 2·37); $P < 0·001$).

A radiological healing line was observed in all thirty-three, thirty-one and thirty-four children who were radiographed at 6 weeks in group A, B and C, respectively.

The drug was tolerated safely by the children in the study. Three children reported spilling of 60 000 IU dose accidently during administration which was repeated the same day. Four children in group A had missed daily doses for 2–5 d during the study period, not amounting to treatment discontinuation or exclusion. Among those who completed the follow-up, no child in group B or C reported non-compliance to any dose.

Residual secondary hyperparathyroidism was observed in 14 (4, 5 and 5 in groups A, B and C, respectively; $P = 0·908$) children with mean serum PTH levels of 77·96 (SD 11·8) pg/ml. These children when compared to children with normal PTH at follow-up had higher mean baseline X-ray scores (8·86 (SD 1·88) and 7·83 (SD 2·34); $P = 0·116$), post-intervention X-ray scores (2·29 (SD 1·68) *v.* 2·01 (SD 1·98); $P = 0·626$) and baseline PTH levels (216·89 (SD 135·11) and 162·40 (SD 143·62) pg/ml; $P = 0·182$) with lower baseline serum 25(OH)D levels (8·11 (SD 4·44) and 10·53 (SD 9·68) ng/ml; $P = 0·358$).

WebTable 1 shows the baseline and post-intervention parameters classified as per baseline vitamin D status. Complete radiological healing was noted in 76·5 % children with 25(OH)D levels ≥ 12 ng/ml and 61·6 % with vitamin D deficiency at baseline, $P = 0·14$. Composite healing was comparable between both groups (100 and 94·2 %; $P = 0·32$). The change in X-ray scores (delta score) was comparable across intervention arms within both groups ($P > 0·05$), data not shown.

Three children had serum 25(OH)D levels of 100 ng/ml (two in group C and one in group B). One infant of these three in group C had suppressed PTH (7 pg/ml) without any hypercalciuria or hypercalcaemia. Seven children (five in group A and two in group B) had hypercalciuria without hypercalcaemia or hypervitaminosis D. The UCaCr ratio was normal in all five subjects who were tested on a repeat spot sample performed after 7 d.

Discussion

The present study observed similar efficacy of daily *v.* weekly vitamin D therapy in radiological healing of nutritional rickets in children. Traditionally, Stoss regimen (600 000 IU) or weekly bolus doses have been used for treating rickets in children with good efficacy. Weekly vitamin D doses have been found comparable to single bolus intramuscular doses when used in comparable doses[6,9] or in lower doses of 90 000 or 300 000 IU[2,3].The present

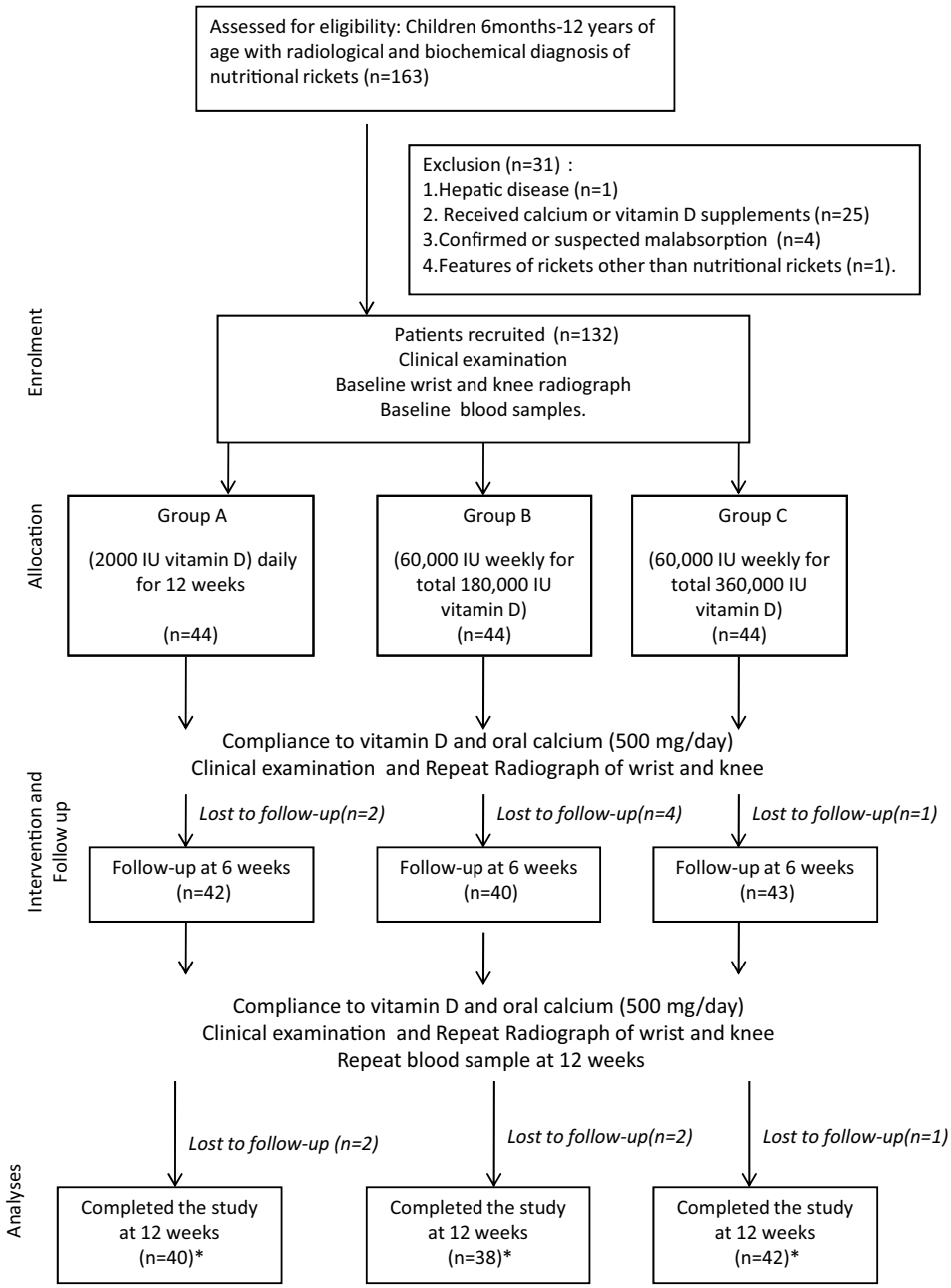

Fig. 1. Flow of the study (intention to treat analyses done with $n = 44$ in all three arms for primary outcome of radiological healing; *limited numbers at the end for per protocol analyses of other parameters).

study likewise observed similar efficacy of cumulative dose of 180 000 IU and 360 000 IU of vitamin D_3.

The overall radiological healing in 65·8 % of subjects at 12 weeks in the present study suggests that healing of rickets may not be complete at 12 weeks[3,20], mandating a longer duration of 4–6 months of treatment and follow-up. Similar observation of incomplete healing at 12 weeks was reported earlier after 600 000 IU of vitamin D_3[21]. In contrast, a higher proportion (97 %) of children achieved complete radiological healing at 3 months with an oral dose of 60 000 IU for 10 weeks or 600 000 IU intramuscular bolus[9] that could possibly be because fewer (40 %) subjects had hypocalcaemia as against 73 % subjects in the present study.

Though the radiological scores were comparable across the three groups, the delta change in the radiological score in group A was more than the other two groups suggesting superior skeletal healing with daily doses than with equivalent or a higher dose as Stoss therapy. This is probably due to sustained cholecalciferol concentration in the daily instead of weekly regimen. Such sustained concentration with daily doses of vitamin D as against bolus dose has been earlier reported in infants where repletion of serum 25(OH)D was initially higher with bolus

Table 1. Baseline characteristics of the study population
(Median values and interquartile ranges; mean values and standard deviations)

	Group A (*n* 44)		Group B (*n* 44)		Group C (*n* 44)	
	Mean	SD	Mean	SD	Mean	SD
Age, months*						
Median	14		11·5		12	
IQR	7·5–23·5		7·25–24		8–23·5	
Birth weight, gm	2525	468·38	2501·6	477·3	2600·4	619·04
Weight, kg	9·1	5·4	8·6	4·6	9·8	7·4
Length/height, cm	73·0	14·1	73·7	16·5	76·4	21·7
Dietary Ca intake, mg/d*						
Median	310		250		300	
IQR	218·75–500		150–450		200–500	
Serum calcium, mg/dl	8·08	1·4	7·82	1·7	7·73	1·9
Serum phosphorus, mg/dl	4·02	1·7	4·12	1·6	3·91	1·2
	Median	IQR	Median	IQR	Median	IQR
Serum ALP, IU/ml*	692·0	464·8–1192·0	670·0	483·8–977·8	663·5	325·5–1018·8
Serum 25(OH)D, ng/ml*	7·8	6·0–11·50	9·0	5·0–14·75	7·5	5–13·68
Plasma PTH, pg/ml*	121·95	57·78–275·5	116·95	51·6–271·8	95·9	55·4–221·85
Urinary Ca: creatinine, mg/mg*,†	0·09	0·02–0·27	0·33	0·17–0·54	0·16	0·07–0·35

Data expressed as mean values and standard deviations;
* median (IQR); ANOVA used for comparison of means and Kruskal Wallis used for comparison of medians among three groups; $P > 0·05$ for all variables;
† $n = 12$, 5 and 9 in groups A, B and C, respectively.

Table 2. X-ray scores in daily *v.* weekly vitamin D groups
(Median values and interquartile ranges; numbers and percentages)

	Group A (*n* 44)		Group B (*n* 44)		Group C (*n* 44)		
	Median	IQR	Median	IQR	Median	IQR	*P*
X-ray score baseline	10	6·5–10	8	5·25–10	8	6–10	0·183
X-ray score 6 weeks*	6	3·5–6·8	4	3–8	4	2·8–6	0·362
X-ray score 12 weeks†	1·0	1·0–3	1·0	1·0–4	1·0	1·0–3·75	0·187
Delta X-ray change‡	7	4–9	5	2·25–6	6	4–7	0·019
Complete radiological healing							
n	30		23		26		0·15
%	75 %		60·5 %		61·9 %		

* Data available for 33, 31 and 34 subjects in group A, B and C, respectively; delta X-ray = score at baseline – score at 12 weeks.
† $P < 0·001$ for all paired comparisons within each group for X-ray score at baseline, 6 weeks and 12 weeks on Friedman's two way rank test.
‡ Post-hoc analysis (Dunn-Bonferroni method) showed statistical significance between group A and B ($P = 0·017$) and non-significant between group B and C and C and A on Kruskal Wallis test.

intermittent doses at 1–2 weeks of therapy but became comparable to daily doses at 3–4 months[22]. However, a poorer response with 2000 IU of daily *v.* 600 000 IU intramuscular dose of vitamin D was reported earlier in infants where daily dose was administered only for 4 weeks[23], suggesting the need for longer duration of daily regimen. Children who were younger and had worse metabolic parameters showed higher residual secondary hyperparathyroidism and poorer skeletal healing at 12 weeks in this study indicating the need to continue treatment surveillance in these patients.

Higher serum 25(OH)D levels in group C as against group A and B directly correlated with the dose of vitamin D administered. Similar observation has also been reported in earlier studies[8,24]. There are conflicting reports with regard to serum 25(OH)D levels achieved following daily/weekly supplementation of vitamin D in comparable doses[7,25–28]. Fewer subjects achieved sufficient serum 25(OH)D levels in group B than group A in the present study suggesting better maintenance of serum 25(OH)D blood levels with daily instead of weekly regimen for comparative doses as also reported earlier[22,25,29,30].

The study had the following limitations: (a) enrolled children over a wide age range, (b) urinary Ca excretion studies and repeat X-rays at 6 weeks were done only in a subset of subjects, (c) bone markers for monitoring changes in bone turnover were not performed and (d) detailed dietary assessment of other foods for Ca intake was not recorded as dairy products and eggs were presumably the common sources of Ca in this population. The role of genetic factors, polymorphisms in vitamin D receptor, vitamin D binding proteins and PTH gene in predicting response to vitamin D therapy[31,32] was also not evaluated in the current study.

The compliance with drugs was good in the daily vitamin D group as the RCT design with active follow-up plan was ensured in this study. The study also reported good healing rate with lower dose of vitamin D_3 in daily and bolus regimens which has treatment implications against use of higher doses of oral

Table 3. Biochemical variables post-intervention
(Median values and interquartile ranges; mean values and standard deviations)

	Group A (*n* 40)		Group B (*n* 38)		Group C (*n* 42)		*P*
	Median	IQR	Median	IQR	Median	IQR	
Serum calcium, mg/dl*							
Mean	9·65		9·46		9·7		0·71
SD	1·0		0·9		0·9		
Serum phosphorus, mg/dl*							
Mean	4·88		4·62		4·95		0·63
SD	1·1		1·2		1·1		
Serum ALP, IU/ml	293·0	213·5–353·3	301·5	196·3–364	275·5	167·5–355	0·67
Serum 25(OH)D, ng/ml†	50·0	26·5–66·5	42·1	28·4–54·4	53·5	33·7–71·2	0·045
Plasma PTH, pg/ml	28	12·3–39·2	31·3	19·6–49·9	30·8	12·7–55·7	0·43
Urinary Ca:Cr, mg/mg‡	0·29	0·1–0·42	0·3	0·15–0·76	0·22	0·11–0·36	0·80
Serum 25(OH)D > 20 ng/ml							
n	37		32		40		0·21
%	92·5 %		84·2 %		95·2 %		
Clinical healing							
n	39		35		41		0·38
%	97·5 %		92·1 %		97·6 %		

ANOVA was used for comparison of serum calcium and phosphate; Kruskal Wallis test used for comparison of serum ALP, 25(OH)D, PTH and urinary Ca:Cr.
* Mean value and standard deviations.
† Post-hoc analysis (Dunn-Bonferroni method) of serum 25(OH)D levels showed statistical significance between group B and C ($P = 0·038$) and non-significant between group B and A and C and A.
‡ $n = 12$, 5 and 9 in groups A, B and C, respectively.

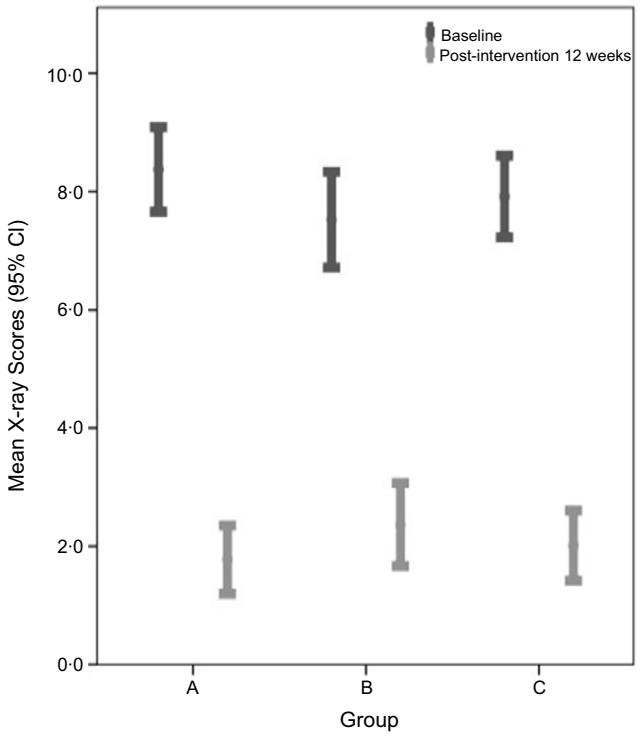

Fig. 2. Bar diagram for X-ray scores (mean and 95 % CI) at baseline and after intervention compared as per vitamin D dosing in groups (a) 2000 IU/d; (b) 180 000 IU cumulative; (c) 360 000 IU cumulative.

vitamin D. The nanoemulsified preparations were easy to administer and have been documented with higher bioavailability than conventional fat soluble preparations earlier[33]. However, higher cost of these nanoemulsified preparations[34] may limit their use in clinical practice.

To conclude, the present study concluded comparable radiological healing suggesting similar efficacy of daily (with good compliance) *v.* weekly vitamin D therapy in children with nutritional rickets. However, a greater radiological improvement was observed from baseline with daily rather than weekly vitamin D regimen for the same dosage. Complete radiological healing, however, was observed in less than three-fourths of children at 12 weeks indicating need for longer surveillance for complete healing.

Acknowledgements

None.

The investigational drugs were supplied by Society for Endocrine Health of Elderly, Adolescents and Children (SEHEAC), New Delhi, India for the research purpose.

R. K. M. and S. Y.: study conceptualisation and design, manuscript editing and critical revision for intellectual content; A. D., V. D. and P. K. D.: protocol and manuscript drafting and writing, acquisition of data and interpretation; M. B., V. T. and S. A. acquisition of data and interpretation, manuscript writing. All authors agree to be accountable for the content and approve the final content of the manuscript.

R. K. M. procured the drugs for the study through SEHEAC which may be perceived as potential conflict of interest. There is no conflict of interest in (1) study design; (2) the collection, analysis and interpretation of data; (3) the writing of the report and (4) the decision to submit the paper for publication by any of the other authors.

References

1. Chibuzor MT, Graham-Kalio D, Osaji JO, *et al.* (2020) Vitamin D, calcium or a combination of vitamin D and calcium for the

treatment of nutritional rickets in children. *Cochrane Database Syst Rev* **4**, CD012581.
2. Mittal M, Yadav V, Khadgawat R, *et al.* (2018) Efficacy and safety of 90 000 *v.* 300 000 single dose oral vitamin D in nutritional rickets: a randomized controlled trial. *Indian J Endocrinol Metab* **22**, 760–765.
3. Mittal H, Rai S, Shah D, *et al.* (2014) 300 000 IU or 600 000 IU of oral vitamin D3 for treatment of nutritional rickets: a randomized controlled trial. *Indian Pediatr* **51**, 265–272.
4. Cesur Y, Caksen H, Gündem A, *et al.* (2003) Comparison of low and high dose of vitamin D treatment in nutritional vitamin D deficiency rickets. *J Pediatr Endocrinol Metab* **16**, 1105–1109.
5. Soliman AT, El-Dabbagh M, Adel A, *et al.* (2010) Clinical responses to a mega-dose of vitamin D3 in infants and toddlers with vitamin D deficiency rickets. *J Trop Pediatr* **56**, 19–26.
6. Koçyiğit C, Çatl G, İnce G, *et al.* (2017) Can Stoss therapy be used in children with vitamin D deficiency or insufficiency without rickets? *J Clin Res Pediatr Endocrinol* **9**, 150–155.
7. Saluja RK, Dewan P, Gomber S, *et al.* (2021) Low dose depot oral vitamin D_3 *v.* daily oral vitamin D_3 for treating nutritional rickets: a randomised clinical trial. *Br J Nutr* **2021**, 1–6.
8. Emel T, Dokan DA, Erdem G, *et al.* (2012) Therapy strategies in vitamin D deficiency with or without rickets: efficiency of low-dose Stoss therapy. *J Pediatr Endocrinol Metab* **25**, 107–110.
9. Mondal K, Seth A, Marwaha RK, *et al.* (2014) A randomized controlled trial on safety and efficacy of single intramuscular *v.* staggered oral dose of 600 000 vitamin D in treatment of nutritional rickets. *J Trop Pediatr* **14**, 203–210.
10. Munns CF, Shaw N, Kiely M, *et al.* (2016) Global consensus recommendations on prevention and management of nutritional rickets. *J Clin Endocrinol Metab* **101**, 394–415.
11. From Indian Academy of Pediatrics 'Guideline for Vitamin D and Calcium in Children' Committee, Khadilkar A, Khadilkar V, *et al.* (2017) Prevention and treatment of vitamin D and calcium deficiency in children and adolescents: Indian Academy of Pediatrics (IAP) Guidelines. *Indian Pediatr* **54**, 567–573.
12. Gupta P, Dabas A, Seth A *et al.* (2021) Indian Academy of Pediatrics Revised (2021) guidelines on prevention and treatment of vitamin D deficiency and rickets. *Indian Pediatr* **59**, 142–158.
13. Harinarayan CV & Akhila H (2019) Modern India and the tale of twin nutrient deficiency-calcium and vitamin D-nutrition trend data 50 years-retrospect, introspect, and prospect. *Front Endocrinol* **10**, 493.
14. National Institute of Nutrition, Indian Council of Medical Research & Ministry of Health and Family Welfare (2017) Indian Food Composition Tables. http://www.ifct2017.com/frame.php?page=food (accessed July 2021).
15. WHO Multicentre Growth Reference Study Group (2006) Assessment of differences in linear growth among populations in the WHO Multicentre Growth Reference Study. *Acta Paediatr* **450**, 56–65.
16. Indian Academy of Pediatrics Growth Charts Committee, Khadilkar V, Yadav S, *et al* (2015) Revised IAP growth charts for height, weight and body mass index for 5- to 18-year-old Indian children. *Indian Pediatr* **52**, 47–55.
17. Hughes HK & Kahl LK (2018) Blood chemistries and body fluids. In *The Harriet Lane Handbook: a Manual for Pediatric House Officers*, pp.708–720 [HK Hughes & L Kahl, editors]. Philadelphia: Elsevier.
18. Leslie SW & Sajja DH (2019) Hypercalciuria. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK448183/#_NBK448183_pubdet_ (accessed May 2019).
19. Thacher TD, Fischer PR, Pettifor JM, *et al.* (2000) Radiographic scoring method for the assessment of the severity of nutritional rickets. *J Trop Paediatr* **46**, 132–139.
20. Chatterjee D, Gupta V, Sharma V, *et al.* (2014) A reliable and cost effective approach for radiographic monitoring in nutritional rickets. *Br J Radiol* **87**, 20130648.
21. Chatterjee D, Swamy MK, Gupta V, *et al.* (2017) Safety and efficacy of Stoss therapy in nutritional rickets. *J Clin Res Pediatr Endocrinol* **9**, 63–69.
22. Huynh J, Lu T, Liew D, *et al.* (2017) Vitamin D in newborns. A randomised controlled trial comparing daily and single oral bolus vitamin D in infants. *J Pediatr Child Health* **53**, 163–169.
23. Lubani MM, Al-Shab TS, Al-Saleh QA, *et al.* (1989) Vitamin-D-deficiency rickets in Kuwait: the prevalence of a preventable disease. *Ann Trop Paediatr* **9**, 134–139.
24. Marwaha RK, Mithal A, Bhari N, *et al.* (2018) Supplementation with three different daily doses of vitamin D3 in healthy pre-pubertal school girls: a cluster randomized trial. *Indian Pediatr* **55**, 951–956.
25. Tan JK, Kearns P, Martin AC, *et al.* (2015) Randomised controlled trial of daily *v.* Stoss vitamin D therapy in Aboriginal children. *J Pediatr Child Health* **51**, 626–631.
26. Wadia U, Soon W, Chivers P, *et al.* (2018) Randomised controlled trial comparing daily versus depot vitamin D3 therapy in 0–16-year-old newly settled refugees in Western Australia over a period of 40 weeks. *Nutrients* **10**, 348.
27. Pappa HM, Mitchell PD, Jiang H, *et al.* (2012) Treatment of vitamin D insufficiency in children and adolescents with inflammatory bowel disease: a randomized clinical trial comparing three regimens. *J Clin Endocrinol Metab* **97**, 2134–2142.
28. Gordon CM, Williams AL, Feldman HA, *et al.* (2008) Treatment of hypovitaminosis D in infants and toddlers. *J Clin Endocrinol Metab* **93**, 2716–2721.
29. O'Callaghan KM, Taghivand M, Zuchniak A, *et al.* (2020) Vitamin D in breastfed infants: systematic review of alternatives to daily supplementation. *Adv Nutr* **11**, 144–159.
30. Meekins ME, Oberhelman SS, Lee BR, *et al.* (2014) Pharmacokinetics of daily *v.* monthly vitamin D3 supplementation in non-lactating women. *Eur J Clin Nutr* **68**, 632–634.
31. Ray D, Goswami R, Gupta N, *et al.* (2009) Predisposition to vitamin D deficiency osteomalacia and rickets in females is linked to their 25(OH)D and calcium intake rather than vitamin D receptor gene polymorphism. *Clin Endocrinol* **71**, 334–340.
32. Yao P, Sun L, Lu L, *et al.* (2017) Effects of genetic and nongenetic factors on total and bioavailable 25(OH)D responses to vitamin D supplementation. *J Clin Endocrinol Metab* **102**, 100–110.
33. Marwaha RK & Dabas A (2019) Bioavailability of nanoemulsion formulations *v.* conventional fat soluble preparations of cholecalciferol (D3) – an overview. *J Clin Orthop Trauma* **10**, 1094–1096.
34. Chugh PK & Dabas A (2021) Price dispersion of vitamin D Supplements over time: an initiative for prescriber education. *Indian J Endocrinol Metab* **25**, 142–147.