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Randomized Controlled Trial

Effect of high dose vitamin d supplementation on vitamin d nutrition status of pre-pubertal children on anti-epileptic drugs – A randomized controlled trial

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SUMMARY

Background and aims: Patients on long term anti-epileptic drug therapy are prone for Vitamin D deficiency for a myriad of reasons. The aim of this research was to study the effect of high dose vitamin D supplementation on vitamin D nutrition status of children newly started on anti-epileptic drug therapy.
Materials: This randomized controlled trial was conducted in a tertiary care Children's Hospital at New Delhi from November 2011 to March 2013. Eighty three children in the age group 5–10 years newly started on anti-epileptic drugs (AED) were randomized into two groups; group A – the intervention group, to whom 60,000 IU vitamin D3 was given orally/month under direct supervision along with AED for a period of 6 months, and group B- the control group, to whom AED without vitamin D3 was given. Serum 25(OH)D, ionized calcium (iCa), total calcium (tCa), inorganic phosphate (iP), alkaline phosphatase (ALP) and parathyroid hormone (PTH) levels were assayed at baseline and at the end of 6 months and were compared within and between the two groups.
Results: The mean 25(OH)D in Group A was maintained at 6 months follow up [26 ng/ml, 95% CI 20 –34 ng/ml] compared to baseline [25 ng/ml, 95% CI –19 to 33 ng/ml] [p = 0.83]. In group B, there was a significant decrease in 25(OH)D levels at 6 months [13 ng/ml (95% CI 9 ng/ml–17 ng/ml)] compared to baseline [18 ng/ml (95% CI 13–24 ng/ml)] [p = 0.01]. At 6 months, mean serum 25(OH)D was significantly higher in group A as compared to group B (p = 0.005).
Conclusion: To conclude, oral administration of 60,000 IU vitamin D3/month is sufficient to maintain serum 25(OH)D level and prevent development of vitamin D deficiency in children newly started on AED over a period of 6 months. Non supplementation leads to the lowering of serum 25(OH)D in these children.
Trial Registration Number: CTRI/2017/08/009234.

1. Introduction

Vitamin D deficiency is recognized as a worldwide problem across all age groups [1–6]. The prevalence of vitamin D deficiency in children from India is reported to be in the range of 43–97% [7–11]. Patients on long term anti-epileptic drugs (AED) are more prone to Vitamin D deficiency [12,13].The exact mechanism for adverse bone effect of anti-epileptic drugs (AED) has not been determined, although many factors are known to influence and modify these effects. Vitamin D deficiency associated with phenytoin, carbamazepine, phenobarbitone and primidone use is likely mediated by PXR (pregnane X receptor). It is shown that AEDs lead to the upregulation of 25-hydroxyvitamin D 3 [25(OH)D]-24-hydroxylase (CYP24) gene expression through the activation of PXR [14]. CYP24 is a mitochondrial enzyme responsible for inactivating vitamin D metabolites. Vitamin D deficiency leads to impaired

Abbreviations: AED, anti-epileptic drugs; ALP, Alkaline phosphatase; ECLIA, electrochemiluminiscence; iCa, ionized calcium; iP, inorganic phosphate; PTH, Parathyroid hormone; tCa, total calcium.

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calcium absorption from the intestine. Ensuing hypocalcemia triggers release of parathyroid hormone which acts on the bone to release the calcium stores, thus leading to bone loss. Valproic acid, an enzyme inhibitor, accelerates bone loss by activating the osteoclasts directly [15] while phenytoin decreases the intestinal absorption of calcium [16].

Various Authorities have recommended different dosages of Vitamin D supplementation for different age groups. Most of them have recommended 400 IU – 600/day for infants and children for general health [17]. The Endocrine Society, USA recommends an intake of 3000–6000 IU/day vitamin D for individuals on drugs affecting vitamin D metabolism such as AED [18]. The Indian Council of Medical Research (ICMR), recommends a RDA of 400 IU/day in populations with minimal sun exposure while there are no recommendations for individuals at higher risk of vitamin D deficiency [19]. There are very few studies on vitamin D supplementation in children on AED. The present study assesses the efficacy of high dose oral vitamin D_3 supplementation (60,000 IU/month) in children receiving AED in preventing vitamin D deficiency.

2. Materials and methods

This was a randomized controlled trial. Children in age group 5–10 years, with normal nutritional status (BMI within ±2 Z scores of WHO reference standards) [20], newly started on AED in our hospital, or presenting within 2 weeks of AED initiation were enrolled. Children who were non ambulatory, receiving calcium/vitamin D supplements, those with clinical or biochemical evidence of rickets or any chronic disease influencing vitamin D metabolism (chronic kidney disease, chronic liver disease, malabsorption states) were excluded. The cases were randomized using block randomization into two groups – group A – the intervention group, to whom 60,000 IU vitamin D_3 was given orally every month along with the prescribed AED for 6 months and group B- the control group, to whom only the prescribed AED was given. Allocation concealment was achieved using sequentially numbered opaque sealed envelopes. The subjects were followed up monthly for 6 months. Repeat dose of oral vitamin D_3 60,000 IU was given monthly under direct supervision to subjects in group A. Venous blood samples were taken at baseline and at the end of 6 months. A portion of the sample was analyzed immediately for total (tCa) and ionized calcium (iCa), inorganic phosphates (iP) and alkaline phosphatase (ALP). The rest of the blood sample was allowed to clot and then centrifuged at 3000 rpm for 5 min. Supernatant serum was removed and was stored in a deep freezer at −80 °C for subsequent assay of serum PTH and 25(OH)D. Serum 25(OH)D was measured using electrochemiluminiscence (ECLIA, COBAS, Roche Diagnostics). Serum PTH was measured using chemiluminescence immunoassay (Beckmann Coulter, Access immunoassay systems). tCa, iCa, ALP and iP were measured using calorimetric assay (COBAS, Roche/Hitachi MODULAR P analyzer).

Vitamin D deficiency was classified as severe deficiency-serum 25(OH)D < 10 ng/ml, deficiency – 10–20 ng/ml, insufficiency – 21–29 ng/ml and sufficiency ≥30 ng/ml.

3. Outcomes

3.1. Primary outcomes

Mean serum 25(OH)D levels in supplemented and not supplemented groups at baseline and at 6 months.

3.2. Secondary outcome variables

Mean serum tCa, iCa, iP, ALP and PTH levels in both the groups at baseline and at 6 months.

3.3. Sample size and statistical analysis

The expected prevalence of severe vitamin D deficiency in children on AED therapy was determined to be 56% [21]. High dose vitamin D supplementation was expected to reduce the prevalence to half (28%) [21].The expected sample size to detect this difference with a confidence of 95% with a power of 80% was 50 in each group. For this study, a sample size of 40 in each group was taken derived from the total number of children meeting inclusion criteria expected to attend the hospital within the proposed study period.

The data were analyzed by statistical software SPSS version 17.0. The normality of biochemical variables was tested by Kolmogrov Smirnov test and it was found that except for 25(OH)D and PTH, all other biochemical variables were normally distributed. Thus, the log base 10 transformation was applied for 25(OH)D and PTH to fulfill the normality condition. Two factor repeated measures ANOVA was applied to compare inter and intra group comparison for all biochemical variables. Fischer's exact test was used for comparison of proportion of children with hypocalcemia, hypophosphatemia and raised ALP. Pearson correlation was used to find the strength of correlation between 25(OH) D and other biochemical variables.

3.4. Ethics

The protocol was approved by the Ethics Committee for Human Research (ECHR), Lady Hardinge Medical College, New Delhi. The trial was registered with Clinical Trials Registry India (CTRI) – CTRI/2017/08/009234.

4. Results

Of the 83 subjects enrolled, the data of 64 children (35 in group A and 29 in group B) followed up for 6 months were analyzed (Fig. 1). The baseline clinical and biochemical characteristics were similar in both the groups (Table 1).

5. Biochemical parameters in study subjects at baseline and follow-up

5.1. Serum calcium: total and ionic

The mean serum total calcium (tCa) in group A and group B at baseline were similar. At 6 months follow up tCa level in group A showed a significant increase ($p = 0.001$), while in group B it showed a significant fall ($p < 0.001$) from baseline. At 6 months, mean tCa in Group A was significantly higher compared to group B ($p = 0.009$). Similar trend was observed in mean serum iCa levels (Table 2).

5.2. Serum inorganic phosphate

Mean serum iP of group A and group B patients at baseline was similar ($p = 0.29$). There was no change in the iP levels in group A or group B at follow up ($p = 0.06$ and $p = 0.56$ respectively) as compared to baseline and no difference between the 2 groups at 6 months follow up ($P = 0.78$).

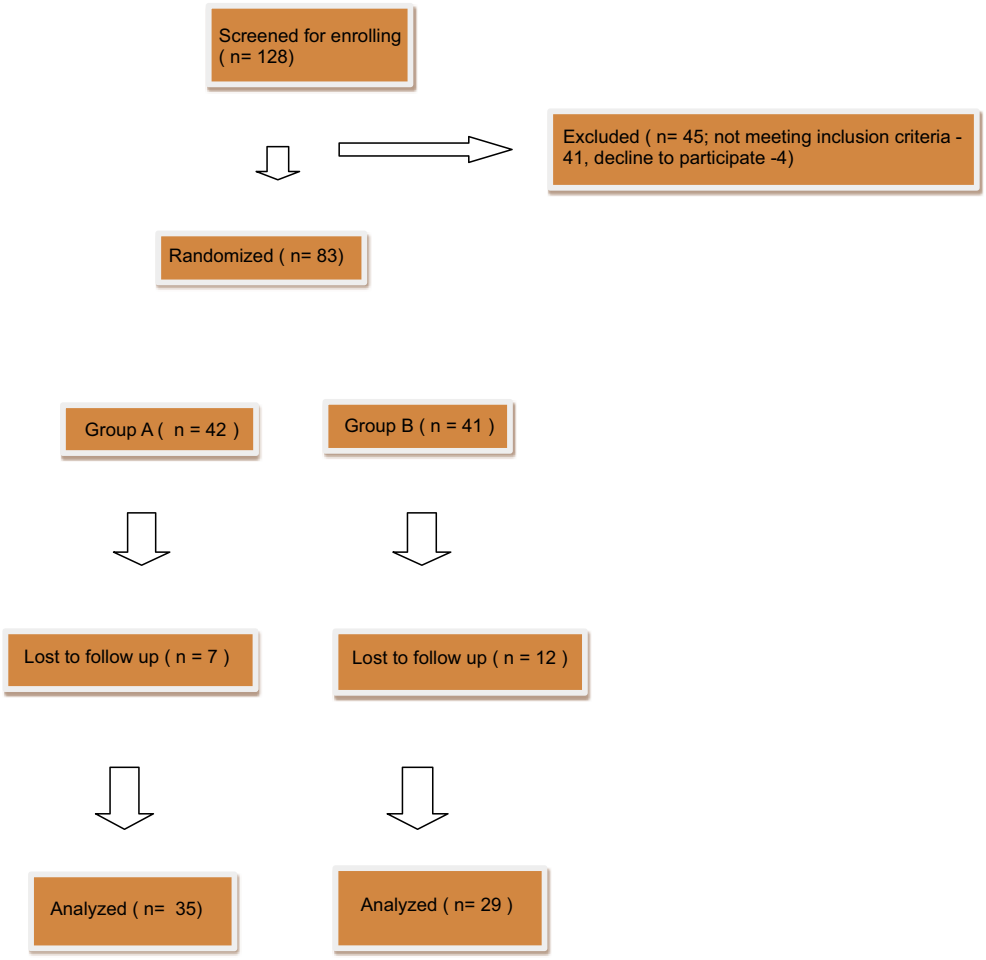

Fig. 1. Study flow.

Table 1
Baseline characteristics of study subjects.

Variable	Group A (n = 35) Mean ± SD	Group B (n = 29) Mean ± SD
Age(Mean ± SD months)	95 ± 20	95 ± 2 0
Male/Female	24/11	23/6
AEDs n(%)		
Phenytoin	21 (60%)	16 (55%)
Valproate	5 (14%)	8 (28%)
Carbamazepine	8 (23%)	2 (7%)
Multiple AED's	1 (3%)	3 (10%)

5.3. Serum ALP

The mean serum ALP level of group A and group B patients was similar at baseline (p = 0.35). At 6 months, the levels increased significantly in group B (P = 0.008), while there was no change in group A (p = 0.99). Mean ALP levels were significantly higher in group B compared to group A at 6 months follow up (p = 0.02) (Table 2).

5.4. Serum parathyroid hormone levels (PTH)

The mean serum PTH level of group A and group B at baseline was similar (p = 0.45). At 6 months follow up, there was no significant increase in the PTH levels in group A (p = 0.79), while it showed a significant increase in group B (p = 0.004). Mean PTH level in group B was significantly higher than that in group A at 6 months follow up (p = 0.01) (Table 2).

5.5. Serum 25(OH)D

The mean serum 25(OH)D of group A and group B at baseline was similar (p = 0.10). While there was no change in the mean 25(OH)D level in group A at 6 months (p = 0.83), in group B there was a significant decrease (p = 0.01). At 6 months, mean 25(OH) D was significantly higher in group A as compared to group B (p = 0.005) (Table 2).

At baseline, combined prevalence of 'severe deficiency' (≤10 ng/ml) and 'deficiency' (11–20 ng/ml) in groups A and B were 37% and 45% respectively (p = 0.53). At 6 months follow up, the combined prevalence of 'severe deficiency' and 'deficiency' in group A was 40% and in group B 62%. There was a trend towards an increase in proportion of patients with deficiency and severe deficiency in group B compared to group A at 6 months follow up (p = 0.08) (Table 3).

5.6. Adverse effects

Children who were on group A were screened for hypercalciuria after three monthly doses of Vitamin D3 and after the completion of therapy. None of them had hypercalciuria. Also, children on group B were regular screened for development of clinical rickets.

Table 2
Mean serum total and ionic calcium, ALP PTH and 25(OH) D at baseline and follow up in both the groups.

Variable	Group	Mean ± SD at baseline	Mean ± SD at follow-up	P value (intra-group baseline vs follow up)	P value *(inter group at follow up)
Ionic calcium (mg/dl)	Group A (n = 35)	4.9 ± 0.6	5.1 ± 0.6	**0.009**	**0.001**
	Group B (n = 29)	4.8 ± 0.5	4.4 ± 0.5	**0.000**	
Total calcium (mg/dl)	Group A (n = 35)	10.0 ± 0.9	10.0 ± 0.9	**0.001**	**0.009**
	Group B (n = 29)	9.7 ± 0.9	9.3 ± 0.8	**0.000**	
Serum alkaline phosphatase (U/L)	Group-A (n = 35)	477 ± 172	477 ± 213	0.99	**0.02**
	Group-B (n = 29)	522 ± 206	683 ± 355	**0.008**	
Serum parathyroid levels (pg/ml)	Group-A (n = 35) GM (95% CI)	26.3 (22.0–31.0)	25.5 (20.5–31.6)	0.79	**0.01**
	Group-B (n = 29) GM (95% CI)	29.3 (22.9–37.6)	43.3 (35.0–53.6)	**0.004**	
Serum 25(OH) D (ng/ml)	Group-A (n = 35) GM (95% CI)	25.0 (18.9–33.1)	25.7 (19.6–33.8)	0.83	**0.005**
	Group-B (n = 29) GM (95% CI)	17.9 (13.4–24.0)	12.7 (9.3–17.5)	**0.010**	

Bold P values are statistically significant.

Table 3
Classification of children according to serum 25OH D levels at baseline and at follow up.

	Baseline		Follow up	
Serum 25(OH) D	Group A (n-35) n (%)	Group B (n = 29) n (%)	Group A (n = 35) n (%)	Group B (n = 29) n (%)
<=10 ng/ml (severe deficiency)	3 (9%)	6 (21%)	5 (14%)	9 (31%)
11–20 ng/ml (Deficiency)	10 (29%)	7 (25%)	9 (26%)	9 (31%)
21–29 ng/ml (Insufficiency)	5 (14%)	8 (28%)	4 (11%)	6 (21%)
≥30 ng/ml (Normal)	17 (49%)	8 (28%)	17 (49%)	5 (17%)

None of the children in the group B developed features of rickets during the 6 month study period.

6. Discussion

In this randomized controlled trial we have studied the serum levels of 25(OH)D and other biochemical markers of bone turn over in children newly started on AED, supplemented them with 60,000 IU vitamin D_3 orally per month for 6 months and compared biochemical parameters in supplemented and unsupplemented children at baseline and follow up.

We found that serum 25(OH)D in the supplemented group was maintained at 6 month follow up while that the control group showed a significant fall. The supplemented group maintained the PTH and ALP levels and had a significant increase in serum tCa and iCa levels. The unsupplemented group had a fall in tCa and iCa and increase in ALP and PTH levels at 6 months. The proportion of subjects with vitamin D deficiency remained similar to baseline in supplemented but showed a significant increase in the unsupplemented group.

6.1. Serum 25(OH)D level at baseline and follow-up

At baseline the mean serum 25(OH)D levels in the subjects was 22 ng/ml. This is similar to our previously reported level of 25 ng/ml in healthy children (6 months–5 years) [22]. In this study, 41% of the enrolled subjects had vitamin D deficiency at baseline. Several other studies conducted in India in the pediatric age group, have shown the prevalence of vitamin D deficiency in the range of 73%–93% [7,11].

In the unsupplemented group B the mean 25(OH)D levels showed a fall of 30% from baseline. A similar fall of 22% in 25(OH)D level was reported by Misra et al. in children newly initiated on carbamazepine therapy over a period of 6 months [23]. Other authors have also reported the effects of AED on biochemical markers of bone turnover [12,13,24]. The mean 25(OH)D of unsupplemented subjects after 6 months of AED therapy in our study is similar to that observed by Nettekoven et al. (Germany) [12] and lower than that observed by Shellhaas et al. (United States Of America) [13]. In our study all the children were on older AEDs (phenytoin, valproate, phenobarbitone and carbamazepine), while, many subjects in study by Shellhaas et al. were on newer generation AEDs which are reported to have little effect on bone metabolism.

In contrast to the unsupplemented group, the mean 25(OH)D in the supplemented group did not show a significant change as compared to baseline at 6 months follow up. In a study conducted by Mikati et al. on children on AED therapy, the mean serum 25(OH) D level rose by 30% in children supplemented with 400 IU/day and by 54% in those supplemented with 2000 IU/day for a period of 1 year [21]. However, the baseline 25(OH)D in their study was much lower than the level in our subjects at enrollment. Subjects with a low baseline 25(OH)D level are reported to experience a higher increase in 25(OH)D level after supplementation with vitamin D as compared to subjects with a higher baseline level. Also, Mikati et al. continued supplementation for 1 year, as against 6 months in our study. Offermann et al. also observed that a dose of 20,000 IU and 40,000 IU/month given for a period of 9 months was sufficient to raise the 25(OH)D levels to normal levels [24].

We observed that the proportion of children with vitamin D deficiency increased from 45% to 62% at end of the 6 month period in the unsupplemented group. Prevalence of vitamin D deficiency in children on AED is reported to be 76% by Nettekoven et al. and 25% by Shellhaas et al. In our study, supplementation while maintaining serum 25(OH)D level, did not decrease the prevalence of vitamin D deficiency, which remained at 40%, thus indicating that a daily vitamin D equivalent of 2000 IU was not enough to treat a pre-existing vitamin D deficiency.

6.2. Other biochemical parameters at baseline and at follow up

Levels of markers of bone turnover such serum tCa and iCa, ALP and PTH were also adversely affected in the non supplemented

group at the end of 6 months, while they remained maintained in the supplemented. Similar observations have been made by Nettekoven et al. and Offermann et al. Thus, our results show that even children on a single AED given for 6 months develop biochemical parameters indicating adverse influence on bone health. A longer duration of therapy and use of multiple AED is likely to exaggerate these effects. None of the supplemented subjects developed hypercalcemia, a concern with high dose vitamin D therapy.

6.2.1. Strengths and limitations of this study

This study was a randomized controlled trial on a subject where limited data exits. Vitamin D was given under direct supervision, thus ensuring 100% compliance with therapy. Major limitations of our study are the small sample size and a high loss to follow-up. Also, though non-significant statistically there was some difference in the baseline mean 25(OH)D levels between the two groups, with the 25(OH)D level being less in the control arm compared to that in the intervention arm. We could not interpret the effect of polytherapy on the level of 25(OH)D since the number of patients in this group was very few. Also, our follow up was for only 6 months while most patients need AEDs for a longer duration. Whether this dose of vitamin D is sufficient for a longer duration of AED therapy as well is not addressed by us.

7. Conclusions

Oral administration of 60,000 IU vitamin D_3/month of is safe and is sufficient to maintain serum 25(OH)D level and prevents development of vitamin D deficiency in children newly started on AED over a period of 6 months. It also improves serum calcium and maintains serum ALP and PTH levels. However, it does not decrease prevalence of vitamin D deficiency as compared to baseline. Hence, a higher dose might be required to normalize the 25(OH)D levels in these vulnerable children. Non supplementation leads to the lowering of serum 25(OH)D and calcium, elevation of serum ALP and PTH and an increase in prevalence of vitamin D deficiency.

Financial disclosure

None.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2018.11.007.

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